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Monitoring and risk assessment of pesticide residues in agricultural / horticultural commodities

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ABSTRACT: A study on monitoring and risk assessment of pesticide residues in agricultural/horticultural commodities revealed that out of 33 samples detected with pesticides, 22 samples showed presence of multiple pesticides and most of these were not having label claim/approval for use in India by CIB&RC in that specific commodity. Chlorpyriphos was the most frequently detected insecticide followed by profenophos. None of the detected pesticides in commodities monitored during the study period resulted in an in take of >50 per cent of ARfD value which indicated that their consumption does not cause acute health risk. Among the different agricultural/horticultural commodities like cardamom, cumin seed and curry leaf, the highest detected level of pesticides *viz*., lambda cyhalothrin and ethion in cardamom, profenophos in cumin seed and chlorpyriphos, profenophos and ethion in curry leaf exceeded 4 per cent of ADI value, which was considered as a margin indicating chronic health risk. Among the different pesticides studied, profenophos was present in levels of the ADI which represented a high level of chronic health risk to consumers. The results call for an investigation into the levels of pesticide residues in cardamom, cumin seed, curry leaf and for tighter regulation and regular monitoring by government and industry.

Key words: Agricultural/horticultural commodities, pesticide, residues, risk assessment

INTRODUCTION

Pesticides are used globally for the protection of food, fibre, feed and human health. If the

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credits of pesticides include enhanced economic potential in terms of increased production of food and fibre and amelioration of vector borne diseases, their debits have resulted in serious health implications to man and his environment. In India, where meeting food demand is a big challenge, use of chemicals like pesticides, antibiotics and fertilizers are unavoidable inputs to ensure a sustained production of food grain to meet the increasing demand.

Food and health authorities around the world are continuously monitoring pesticide residues in different agricultural commodities by setting Maximum Residue Levels (MRLs) of pesticide in foods. The results of monitoring studies focus on the proper use and exact concentration of pesticides. MRLs encourage food safety by restricting the concentration of a pesticide residue permitted on a commodity (Claeys *et al*., 2011).

Among the different sources of exposure to pesticides, food appears to be the most significant as pesticide residues were constantly detected in some of the raw agricultural commodities (Mathew *et al*., 2012). Therefore, assessing the risk of pesticide residues in agricultural commodities intended for human consumption is necessary. The potential health risks from acute and chronic dietary exposure to pesticides can be assessed by comparing the daily intake with the toxicological reference dose *ie*. Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD) (WHO, 1997).

The aim of the present study is to monitor the presence of residues of pesticides (organochlorines, organophosphates and synthetic pyrethroid group) commonly used on agricultural commodities. The results of the monitoring data in combination with food consumption data were taken into consideration to evaluate whether the ADI and ARfD of pesticides through the consumption of agricultural commodities is a cause of toxicological concern according to the recommended dose by the Food and Agriculture Organization (FAO) and World Health Organization (WHO).

MATERIALS AND METHODS

Sampling

One sample each of agricultural commodities like parboiled rice, raw rice and basmathi rice, branded rice flour, wheat, atta and maida (one kilogram each), cardamom and cumin seed (500 g each), capsicuam, okra and curry leaf (2 kilogram each) were collected from Thiruvanathapuram district at monthly intervals for a period of six months (January 2012 - June 2012). Samples were analyzed within 24 hr and stored at 4°C until the moment of extraction.

Chemicals

Certified Reference Materials (CRM) of different pesticides used in the present study having purity ranging from 95.10 to 99.99 per cent were purchased from M/s Sigma Aldrich and stored in a freezer at low temperature, with light and moisture excluded. Standard stock solutions

were made by dissolving each analytical standard in distilled acetone and diluted with n-hexane: toluene (1:1) to obtain a stock solution of 1000 mg L^{-1} The stock, intermediate standards and working standard solutions were prepared and stored at "20°C until analysis.

Analytical procedure

The whole quantity of each commodity is blended and a representative sample of 25 g (parboiled rice, raw rice, basmathi rice, wheat, leaflets removed from curry leaf, capsicum and okra), 5 g (rice flour, atta and maida) and 8 g (cardamom and cumin) were analyzed for the presence of pesticide residues following QuEChERS method.

a. Rice and wheat grains

Twenty five gram of coarsely ground samples of rice and wheat grain were taken in 200 ml centrifuge. To this, 25 ml distilled water and 50 ml acetonitrile was added and the mixture was placed on a mechanical shaker for 30 min at 1200 strokes min^{-1}. A total of 12-15 g of activated sodium chloride was added. After centrifuging at 2500 rpm for 4 min, 16 ml supernatant was transferred into a 50 ml centrifuge tube containing 2.0 g sodium sulphate and 2.0 g magnesium sulphate and vortexed at full speed for 30 s and then centrifuged for 5 min at 2500 rpm. Twelve ml of upper organic phase was transferred to another 15 ml centrifuge tube containing 0.75 g and 0.10 g each of anhydrous magnesium sulphate and Primary Secondary Amine (PSA) respectively. After centrifuging at 2500 rpm for 5 min, an aliquot of 4.0 ml supernatant was concentrated using Turbovap (50^0C) and final volume was made up to one ml using n-hexane and analyzed by Gas Chromatograph.

b. Rice flour, atta and maida

Five gram of rice flour, atta and maida samples were taken in 50 ml centrifuge tubes in four replicates each and soaked in 10 ml of water for 10 min. Then, the samples were extracted using 15 ml acetonitrile in a 50 ml centrifuge tube with 150 µL of acetic acid. Subsequently, 6.0 g anhydrous magnesium sulphate and 1.5 g sodium acetate were added, immediately shaken for one min and then the extract was centrifuged at 1500 rpm for 5 min. Ten ml of the upper layer was transferred to a 15 ml centrifuge tube containing 500 mg of Primary Secondary Amine (PSA) and 1.5 g of anhydrous magnesium sulphate. The centrifuge tube was shaken for 30 seconds followed by centrifugation for one min at 1500 rpm. Six ml from the upper layer was taken and concentrated to dryness using Turbovap (50^0C) and final volume was made up to one ml using n-hexane and analyzed by GC.

c. Cardamom and Cumin seed

Eight gram of coarsely ground cardamom and cumin seed samples taken in 50 ml centrifuge tubes To this, 4.0 g activated magnesium sulphate and 1.0 g sodium chloride were added. Then 10 ml of chilled distilled water (4^0C) and 15 ml of acetonitrile were added and the samples

were shaken for one min in a vortex and centrifuged at 3500 rpm for 2 min. A dispersive solid phase extraction cleanup process was carried out by transferring the supernatant (6.0 ml) to a centrifuge tube (15 ml) containing 1.0 g magnesium sulphate (hydrated) and 0.30 g PSA (Primary Secondary Amine) and 0.50 g florisil. These tubes containing the supernatant and the reagents were shaken for a few seconds followed by centrifugation at 3500 rpm for 2 min. The cleaned supernatant extract was evaporated to dryness using Turbovap (50^{o}C). The dry residue was reconstituted to one ml with a mixture of n-hexane: acetone (7:3, v/v basis) and analyzed in a Gas Chromatograph

d. Capsicum, curry leaf and okra

Twenty five gram each of blended curry leaf, capsicum and okra were taken in 200 ml centrifuge tubes. A volume of 50 ml acetonitrile was added to the mixture and then homogenized at 14000 rpm for one min. Ten gram of sodium chloride was added to the mixture and centrifuged at 2000-2500 rpm for 4 min. From this, 16 ml supernatant was transferred to a 50 ml centrifuge tube containing 6.0 g sodium sulphate and vortexed. A total of 12 ml supernatant was then transferred to a 15 ml centrifuge tube containing 1.2 g magnesium sulphate and 0.2 g Primary Secondary Amine (PSA) and vortexed again at full speed for 30 s and centrifuged at 2500 rpm for 3 min. After that, 4.0 ml of upper layer was evaporated to dryness using Turbovap at 50^{0}C. The dry residue was reconstituted to one ml using n-hexane and analyzed in a Gas Chromatograph.

Instrument analysis

Gas Chromatograph – (Shimadzu GC 2010 A) equipped with ^{63}Ni Electron Capture Detector (ECD), fitted with DB-5 capillary column (dimethyl polysiloxane, 30m x 0.25mm i.d. x 0.5µm film thickness) was used for the analysis. Ultra high Purity (99.999 %) nitrogen was used as carrier gas with a column flow rate of 0.79 ml min^{-1} and linear velocity 26.00 cm S^{-1}. A column temperature programme was developed to get proper separation of all pesticides used in the analysis. The operating parameters of the instrument were: oven temperature 170^{o}C (5 min) '!1.5^{o}C min^{-1} '!220^{o}C (10 min) '!4^{o}C min^{-1}' !280^{o}C (7 min), injection port at 250^{o}C and detector at 300^{o}C and the total run time as 70 min. and split ratio of 1: 10.

Residue Quantification

$$\text{Pesticide residue in substrate (mg kg}^{-1}) = \frac{\text{Peak area of sample} \times \text{Concentration of standard injected} \times \text{Final volume of sample injected x Dilution Factor}}{\text{Peak area of standard x Volume of sample injected}}$$

RESULTS AND DISCUSSION

Pesticide residues in agricultural/horticultural commodities

In this study, Multi Residue Methods (MRM) for pesticide residue analysis in agricultural/ horticultural commodities was validated by conducting recovery studies. The results demonstrated that the method followed had a satisfactory analytical performance in terms of selectivity and linearity. Good linearity was found within the range of 0.01-0.5 mg kg^{-1} for the pesticides belonging to OC, OP and SP insecticide groups. Satisfactory recoveries and RSDs were achieved for all the pesticides evaluated even at the lowest level of fortification. The mean recovery of all the pesticides under study were in the range 70 - 110 per cent and the repeatability of the recovery results, as indicated by the RSD < 20 % confirmed that the method is sufficiently reliable for pesticide residue analysis in different agricultural commodities.

Monitoring study revealed the presence of 14 different pesticides (Table 1) *viz*., malathion, chlorpyriphos, fenvalerate, methyl parathion, cypermethrin, quinalphos, profenophos, bifenthrin, lambda cyhalothrin, ethion, alpha endosulphan, triazophos, fenpropathrin and beta cyfluthrin belonging to organophosphate (7), synthetic pyrethroid (6) and organochlorine (1) group in the samples analysed.

Among the different pesticides, chlorpyriphos (O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) was the most frequently detected insecticide and it may be due to its preference by farmers because of its broad spectrum activity as insecticide, acaricide and nematicide. Profenophos (O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate) has been the second frequently detected organophosphate (19 samples) pesticide, registered for use only in cotton and tea in India. Because of its translaminar, ovicidal and growth stimulating nature, it is widely used by the farmers. Being a pesticide banned for sale and use in Kerala state, its presence in curry leaf sample to the tune of 0.033 to 25.63 mg kg^{-1} has to be viewed seriously (AICRP(PR), 2012).

Considering pesticide groups, it may be concluded that insecticides belonging to organophosphate group predominated over synthetic pyrethroid and organochlorine compounds. This trend is supported by the consumption pattern of pesticides which also indicated greater use of organophosphates when compared with synthetic pyrethroids and organochlorines (Adityachaudhury *et al*., 1997).

The study revealed the presence of pesticide residues like chlorpyriphos, malathion, methyl parathion, quinalphos, cypermethrin and fenvalerate in cereals like basmathi rice and wheat (Table 1). Among the different insecticides, chlorpyriphos and malathion were the major contaminants. Malathion, chlorpyriphos, dichlorovos (DDVP), fenitrothion and synthetic pyrethroids like cypermethrin are reported to be misused widely as grain protectants during storage (Zhang *et al*., 2010). Basmathi rice being the most expensive brand of rice, grain

Table 1. Pesticide residues in various agricultural/horticultural commodities collected from market during January - June 2012

Commodity	Insecticide detected	Concentration (mg kg^{-1})	Commodity	Insecticide detected	Concentration (mg kg^{-1})
Parboiled rice	ND	-	Atta	ND	-
Raw rice	ND	-	Maida	ND	-
Basmathi rice	Malathion	0.06 -0.08	Rice flour	ND	-
	Chlorpyriphos	0.025			
	Fenvalerate	0.052			
	Methyl parathion	0.046			
	Cypermethrin	0.011			
Wheat	Malathion	0.024 -0.19	Okra	Malathion	0.038
	Chlorpyriphos	0.047 -0.31		Profenophos	0.121
	Quinalphos	0.039 – 0.046			
	Methyl parathion	0.065			
Cumin seed	Chlorpyriphos	0.04-0.270	Capsicum	Chlorpyriphos	0.024 -0.047
	Profenophos	0.488-1.45		Profenophos	0.033
	Quinalphos	0.139			
	Alpha endosulphan	0.115-0.135			
Cardamom	Chlorpyriphos	0.057-0.353	Curry leaf	Chlorpyriphos	0.014-1.34
	Profenophos	0.139-0.954		Profenophos	1.62-25.63
	Quinalphos	0.137-2.044		Quinalphos	0.209-0.259
	Cypermethrin	0.061-0.461		Alpha endosulphan	0.015
	Lambda cyhalothrin	0.058-0.364		Triazophos	0.36-1.58
	Ethion	0.344		Cypermethrin	0.12-1.44
	Bifenthrin	0.106		Fenpropathrin	0.14-0.143
				Beta cyfluthrin	0.08
				Methyl parathion	0.113
				Malathion	0.078-0.439
				Ethion	1.15
				Bifenthrin	0.104

ND – Not Detected

protectants are more likely to be applied which might have resulted in pesticide residue. In contrary to this, none of the samples of raw rice, parboiled rice, rice flour, atta and maida showed the residues of any pesticide. Fractionation of residues in different wheat and rice portions (bran, germ, semolina, grout and flour) during milling and polishing could be the reason for the absence of pesticide residues in atta, maida and rice flour. Our findings are in line with those of Uygun *et al.* (2005) reported reduction of malathion residues about 95 per cent in wheat through milling (to flour).

The data generated through monitoring studies in cardamom have established the overdependence and abuse of pesticides as evident from the range of chemically different pesticides like chlorpyriphos, quinalphos, profenophos, cypermethrin, lambda cyhalothrin, ethion and bifenthrin that were identified and quantified. Presence of multiple residues has undoubtedly established rotational spraying of these pesticides directly on capsules. All the cardamom samples analyzed during the study period contained quinalphos residues above MRL fixed by FSSAI and all the insecticides detected except quinalphos were not at all registered for use in cardamom. Usha (2007) reported that there has been an increase in the pesticide consumption in cardamom during the last ten years and the results of a survey showed an unscientific and non judicious use of pesticides by farmers in Kattapana block of Idukki district. Cumin seed samples tested in the present study were found to be frequently contaminated with residues of profenophos, chlorpyriphos, quinalphos and alpha endosulphan for which no FSSAI MRL exists which means none of the pesticides detected were registered for use in this commodity.

Monitoring of pesticide residues in curry leaf revealed the presence of 12 different pesticide molecules *viz*., chlorpyriphos, quinalphos, profenophos, triazophos, methyl parathion, cypermethrin, alpha endosulphan, malathion, fenpropathrin, cyfluthrin, bifenthrin and ethion at varying levels. A level as high as 25.63 mg kg^{-1} of profenophos was detected in one sample of curry leaf. Eventhough it is not registered for use in curry leaf, it is widely used against psyllids, citrus butterfly and citrus leaf roller in curry leaf because of its high bioefficacy, translaminar and growth promoting action.

Most of the samples tested in the present study had multiple residues with some samples containing three to six pesticides. However, most of these detected pesticides were not registered for use in India by CIB (RC) on that specific commodity. All the cardamom, cumin seed and curry leaf samples showed multiple pesticide residues at varying levels. None of the pesticides detected in curry leaf and cumin seed were registered for use in these commodities. Another important factor to consider is the presence of pesticides like methyl parathion, profenophos and endosulphan in basmathi rice, cardamom, cumin seed, curry leaf, capsicum and okra samples tested which were banned for sale and use in Kerala state (Report of Government of Kerala, 2011).

Table 2. Monitoring and risk assessment of pesticides detected in different agricultural/horticultural commodities

Commodity	*Amount consumed per day (g/day/ person)	Pesticides detected mg kg^{-1})	Highest residue level (mg kg^{-1})	Average daily intake (mg kg^{-1} bodyweight)	**ADI (mg kg^{-1} body weight)	% of ADI based on highest residue level	**ARfD (mg kg^{-1} bw)
Basmathi rice	275.00	Malathion	0.08	3.6×10^{-4}	0.03	0.26	0.3
		Methyl parathion	0.046	2.1×10^{-4}	0.003	1.53	0.3
		Chlorpyriphos	0.025	1.14×10^{-4}	0.01	0.25	0.1
		Cypermethrin	0.011	5.04×10^{-5}	0.05	0.02	0.2
		Fenvalerate	0.052	2.38×10^{-4}	0.02	0.26	NA
Wheat	172.80	Malathion	0.19	5.4×10^{-4}	0.03	0.63	0.3
		Methyl parathion	0.065	1.8×10^{-4}	0.003	2.16	0.3
		Chlorpyriphos	0.31	8.9×10^{-4}	0.01	3.1	0.1
		Quinalphos	0.039	1.12×10^{-5}	NA	-	NA
Cardamom	0.8	Chlorpyriphos	0.353	4.7×10^{-9}	0.01	3.53	0.1
		Quinalphos	2.044	2.72×10^{-5}	NA	-	NA
		Profenophos	0.954	1.27×10^{-6}	0.03	3.18	1.0
		Lambda cyhalothrin	0.364	4.85×10^{-6}	0.005	7.28	0.0074
		Cypermethrin	0.461	6.14×10^{-6}	0.05	0.92	0.2
		Ethion	0.344	4.58×10^{-6}	0.002	17.20	0.015
		Bifenthrin	0.106	1.41×10^{-6}	0.015	0.70	0.03
Cumin seed	0.8	Chlorpyriphos	0.27	3.6×10^{-6}	0.01	2.7	0.1
		Quinalphos	0.139	1.85×10^{-6}	NA	-	NA
		Profenophos	1.45	1.33×10^{-5}	0.03	4.83	1.0
		Alpha endosulphan	0.135	1.80×10^{-5}	0.006	2.25	0.02
Capsicum	8.7	Profenophos	0.033	4.7×10^{-6}	0.03	0.11	1.0
		Chlorpyriphos	0.047	6.81×10^{-6}	0.01	0.47	0.1

Commodity	*Amount consumed per day (g/day/ person)	Pesticides detected mg kg^{-1})	Highest residue level (mg kg^{-1})	Average daily intake (mg kg^{-1} bodyweight)	**ADI (mg kg^{-1} body weight)	% of ADI based on highest residue level	**ARfD (mg kg^{-1} bw)
Okra	4.1	Profenophos	0.121	8.26×10^{-6}	0.03	0.40	1.0
		Malathion	0.038	2.59×10^{-6}	0.03	0.12	0.3
Curry leaf	2	Chlorpyriphos	1.34	4.46×10^{-5}	0.01	13.40	0.1
		Malathion	0.439	1.46×10^{-5}	0.03	1.46	0.3
		Quinalphos	0.259	8.63×10^{-6}	NA	-	NA
		Methyl parathion	0.113	3.76×10^{-6}	0.003	3.76	0.3
		Profenophos	25.63	8.54×10^{-4}	0.03	85.43	1.0
		Cypermethrin	1.44	4.8×10^{-5}	0.05	2.88	0.2
		Ethion	1.15	3.8×10^{-5}	0.002	57.50	0.015
		Bifenthrin	0.104	3.46×10^{-6}	0.015	0.69	0.03
		Fenpropathrin	0.143	4.76×10^{-6}	0.03	0.47	NA
		Alpha endosulphan	0.015	5×10^{-7}	0.006	0.25	0.02

*Assuming a 60 kg person, total intake of each commodity is estimated from cluster diets compiled by the Global Environment Monitoring System–Food Contamination Monitoring and Assessment Programme (WHO/GEMS/FOODS) on http://www.who.int/foodsafety/chem/gems/en/index1.html.
** PPDB: Pesticide http://sitem.herts.ac.uk/aeru/footprint/en/index.html.

Risk assessment

Risk assessment was conducted based on monitoring results of the pesticide residue to determine the degree of risk by the detected pesticide residues in samples. WHO has recommended to compare the maximum detected level of pesticide with percentage of ARfD and percentage of ADI for assessing acute and chronic health risk. If pesticides detected resulted in an intake of >50 per cent of percentage of ARfD and >4 per cent of percentage of ADI value, it can be considered to cause acute and chronic health risk (Dalvie and London, 2008)

The highest detected level of the pesticides like lambda cyhalothrin and ethion in cardamom, chlorpyriphos, profenophos and alpha endosulphan in cumin seed and chlorpyriphos, profenophos, malathion, cypermethrin, ethion and bifenthrin in curry leaf exceeded the ARfD values (Table 2). However, none of the detected pesticides resulted in an intake of >50 % of ARfD value which gave an impression of no acute health risk, as per the guidelines of WHO.

Among the different agricultural/horticultural commodities like cardamom, cumin seed and curry leaf, the highest detected level of pesticides like (lambda cyhalothrin and ethion in cardamom, profenophos in cumin seed and chlorpyriphos, profenophos and ethion in curry leaf exceeded >4 % of ADI value fixed for the representative insecticides (Table 2). Among the different pesticides studied, profenophos was present in levels of >85.00 % of the ADI which represented a very high level of a chronic health risk to consumers as indicated by more than 20 times that of the safe margin. So it may be concluded that consumption of cardamom, curry leaf and cumin seed for a longer period of time can cause chronic health risk to consumers. High extent of pesticide residues in agricultural/horticultural commodities calls for improved management of residues at production, post harvest and marketing of food commodities. So, great significance has to be given to simple cost effective strategies like dipping in different decontaminating solutions like 2% tamarind, 2% acetic acid, 2% common salt, washing, cooking and decortications of cardamom capsules to eliminate harmful pesticides which could be practiced by home makers.

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Association of okra *(Abelmoschus esculentus* (L.) Moench) yellow vein mosaic incidence with population of its vectors under Kerala conditions

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ABSTRACT: One hundred and one accessions of okra (*Abelmoschus esculentus* (L.) Moench), collected from various parts of India, were scored for Yellow vein mosaic disease (YVM) incidence and population of two vectors of the disease during summer season during four stages of the crop. The accessions differed significantly for whitefly population during all the three stages of the crop (30, 50 and 70 DAS), while leaf hopper count showed significance only during 50 DAS. Time of infestation differed significantly for white fly at 30 DAS whereas in all other cases, time mean square was non-significant. Correlation coefficients of YVM incidence with vector population computed during different crop stages revealed that morning and evening population of both whitefly and leaf hopper (except for morning population with YVM during final harvest) at 30 DAS had significant association with disease occurrence from 50 DAS to final harvest. Besides, white fly population during both the time at 50 DAS also had influence on YVM incidence during final harvest.

KEYWORDS: okra, *Abelmoschus esculentus,* germplasm, yellow vein mosaic, vectors, whitefly, leaf hopper, *Bemisia tabaci*, *Empoasca devastans*, association, population

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) is an important vegetable all over the world

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having a wide spectrum of uses. Yellow vein mosaic (YVM), the most destructive viral disease of okra, has become a serious limiting factor in the successful cultivation of this crop, which could reduce the yield by 30 to 70 per cent (Duzyaman, 1997). YVM virus belongs to the family *Geminiviridae* and YVM disease is caused by a complex virus consisting of the monopartite begomovirus and a small satellite DNA β component (Jose and Usha, 2003). Disease causes a reduction of leaf chlorophyll and the infected plants become stunted and produce small-sized pale yellow fruits (Gupta and Paul, 2001).

Spread of the virus, causing YVM, by whitefly (*Bemisia tabaci*) was established by Varma (1952) and later reported by many researchers (Ali *et al*., 2000). However, during rainy season, white flies were not common on the crop whereas okra leaf hopper (*Empoasca devastans* (Dist.)) was abundant on the diseased plants (Varma, 1955). Depending upon the stage of crop growth at which infection occurs, yield loss ranged from 50 to 90 per cent (Sastry and Singh, 1974). Any study regarding the magnitude and influence of vector population during various stages of crop growth on YVM disease development could not be found so far. Hence the current study was carried out to find out the interaction of genotypes with the vectors for YVM incidence and also to find out the association of the time of feeding by the vectors with the disease development.

MATERIALS AND METHODS

A germplasm collection of 101 okra varieties / genotypes obtained from various parts of India including known yellow vein mosaic (YVM) resistant varieties, varieties released by Kerala Agricultural University, types from NBPGR Regional Station, Vellanikkara and local collections formed the materials for the study. The genotypes were laid out in Randomised Block Design with three replications and ten plants per treatment per replication at spacing of 60 x 45 cm during summer season to evaluate the resistance of the accessions against YVM. Cultural and manurial practices were followed as per Package of Practices Recommendations of KAU (1996). The experiment was completely devoid of plant protection measures. A local susceptible variety was also grown all around the experimental area to ensure the adequate inoculum for heavy disease incidence as well as vector population. Scoring for disease incidence was done as per the rating scale (Table 1) by Arumugam *et al*. (1975) during four stages of the crop *viz*., 30 days after sowing (DAS), 50 DAS, 70 DAS and final harvest. Population of the two vectors of YVM disease *viz*., whitefly (*Bemisia tabaci*) and leaf hopper (*Empoasca devastans*), were recorded on the plants during 30 DAS, 50 DAS and 70 DAS stages of the crop. Since the leaves were dried, vector population could not be noticed during final harvest stage. The lower sides of the top three leaves in each plant were observed and the number of whiteflies and leaf hoppers were counted separately in the morning as well as evening of the same day. ANOVA was carried out for YVM scores taken on morning and evening populations of white fly and leaf hoppers during each crop stage. Two-factor ANOVA was done for various stages to study the interaction effects between genotypes and time for vector populations. Since genotype x time interaction mean square was non-significant, pooled error mean square was used for testing the significance among the genotypes. In

Table 1. YVM disease rating scale in okra

Sl. No.	Symptom	Grade	Disease **Score**
1	No visible symptom characteristic of the disease	Highly resistant	1
2	Very mild symptoms, basal half of primary veins remain green, mild yellowing of anterior half of primary veins, secondary veins and veinlets. Infection is also seen late in the season under field conditions	Resistant	2
3	Veins and veinlets turn completely yellow	Moderately resistant	3
4	Pronounced yellowing of veins and veinlets, 50 % of leaf lamina turn yellow, fruits exhibit slight yellowing	Susceptible	4
5	Petioles, veins, veinlets, and interveinal area turn yellow in colour. Leaves start drying from margin and fruits turn yellow	Highly susceptible	5

order to find out the association between YVM incidence and population of vectors, correlation coefficients were estimated between YVM scores and population of each vector during each crop stage and the results are furnished in Table 4.

RESULTS AND DISCUSSION

i) Interaction between okra genotypes and vectors of YVM

The 101 genotypes of okra were simultaneously scored for YVM incidence and for vector population of both white fly and leaf hopper in the morning and evening at various crop stages (Table 2). The four okra genotypes which could express highly resistant property throughout the crop phase included NBPGR / TCR – 2060 (T34), Parbhani Kranti (T85), Varsha Uphar (T86) and Selection-46 (T91). Interaction between the genotypes and the populations of each vector was examined separately, twice a day during three stages of the crop. During all the crop stages, interaction mean squares were non-significant for both vectors (Table 3).

The genotypes varied significantly for whitefly population during 30 DAS, 50 DAS and 70 DAS stages of the crop. However, leaf hopper count showed significance only during 50 DAS.

Though time of infestation differed significantly for whitefly at 30 DAS, time mean square was non-significant in all other cases.

Table 2. Mean population of vectors on okra genotypes during three crop stages

Tr. No.	Genotype	YVM score				Whitefly						Leaf hopper					
		30 DAS	50 DAS	70 DAS	FH	30 DAS		50 DAS		70 DAS		30 DAS		50 DAS		70 DAS	
						M	E	M	E	M	E	M	E	M	E	M	E
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	NBPGR/TCR-1185	1.3	2.8	3.7	4.7	0.05	0.05	0.03	0.06	0.05	0.10	0.08	0.00	0.12	0.10	0.03	0.00
2	NBPGR/TCR-1943	1.0	3.5	3.7	5.0	0.08	0.16	0.38	0.50	0.08	0.16	0.16	0.08	0.38	0.42	0.08	0.00
3	NBPGR/TCR-1883	1.0	1.8	3.0	4.7	0.12	0.16	0.08	0.00	0.00	0.00	0.21	0.16	0.25	0.16	0.04	0.00
4	NBPGR/TCR-1948	1.0	1.5	2.5	4.0	0.12	0.08	0.29	0.41	0.21	0.25	0.25	0.16	0.42	0.50	0.21	0.41
5	NBPGR/TCR-2145	1.0	2.3	4.5	5.0	0.04	0.08	0.00	0.00	0.00	0.00	0.12	0.08	0.12	0.08	0.00	0.00
6	NBPGR/TCR-1674	1.0	2.8	3.2	4.7	0.04	0.08	0.16	0.00	0.16	0.16	0.20	0.24	0.58	0.65	0.13	0.00
7	NBPGR/TCR-1676	1.0	2.1	3.5	4.7	0.10	0.19	0.16	0.21	0.07	0.14	0.34	0.39	0.26	0.33	0.11	0.21
8	NBPGR/TCR-1581	1.0	3.0	3.3	4.6	0.27	0.21	0.29	0.41	0.06	0.04	0.27	0.00	0.57	0.56	0.18	0.08
9	NBPGR/TCR-1728	1.0	1.5	3.0	5.0	0.24	0.31	0.08	0.16	0.00	0.00	0.33	0.50	0.33	0.33	0.08	0.00
10	NBPGR/TCR-1981	1.0	4.0	4.5	5.0	0.04	0.00	0.32	0.24	0.08	0.00	0.29	0.08	0.61	0.50	0.33	0.16
11	NBPGR/TCR-1722	1.0	2.0	4.6	5.0	0.08	0.00	0.42	0.50	0.00	0.00	0.32	0.31	0.66	0.65	0.25	0.16
12	NBPGR/TCR-1828	1.0	3.8	4.2	5.0	0.32	0.31	0.59	0.50	0.08	0.00	0.31	0.31	0.70	0.83	0.16	0.00
13	NBPGR/TCR-1508	1.0	2.0	2.9	5.0	0.12	0.16	0.04	0.08	0.00	0.00	0.25	0.42	0.12	0.16	0.04	0.00
14	NBPGR/TCR-1507	1.0	2.8	3.6	4.8	0.06	0.11	0.14	0.05	0.03	0.00	0.19	0.16	0.31	0.28	0.06	0.00
15	NBPGR/TCR-2020	1.0	2.0	2.5	3.9	0.16	0.16	0.17	0.33	0.00	0.00	0.16	0.16	0.33	0.16	0.16	0.16
16	NBPGR/TCR-1498	1.0	2.0	3.0	4.8	0.24	0.31	0.38	0.50	0.04	0.00	0.16	0.31	0.42	0.50	0.08	0.00
17	NBPGR/TCR-1569	1.0	1.5	3.0	4.7	0.04	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.16	0.08	0.08	0.08
18	NBPGR/TCR-1533	1.0	1.8	3.2	5.0	0.20	0.31	0.16	0.16	0.04	0.00	0.08	0.08	0.29	0.42	0.13	0.17
19	NBPGR/TCR-1471	1.0	2.5	3.5	5.0	0.31	0.31	0.41	0.50	0.21	0.16	0.37	0.24	0.46	0.50	0.54	0.50
20	NBPGR/TCR-1963	1.1	2.8	3.5	4.5	0.08	0.00	0.24	0.31	0.04	0.08	0.37	0.41	0.46	0.41	0.00	0.00
21	NBPGR/TCR-1524	1.0	3.4	3.6	5.0	0.16	0.31	0.24	0.27	0.04	0.00	0.30	0.04	0.25	0.16	0.10	0.00
22	NBPGR/TCR-1929	1.0	2.3	2.7	4.7	0.00	0.00	0.54	0.58	0.21	0.33	0.29	0.33	0.55	0.67	0.21	0.25
23	NBPGR/TCR-1966	1.0	1.7	2.0	2.6	0.13	0.07	0.27	0.25	0.00	0.00	0.09	0.07	0.24	0.22	0.05	0.00
24	NBPGR/TCR-1998	1.0	2.3	3.0	4.7	0.29	0.41	0.08	0.16	0.16	0.16	0.29	0.41	0.25	0.16	0.25	0.33
25	NBPGR/TCR-1982	1.0	2.0	2.5	4.0	0.00	0.00	0.31	0.31	0.08	0.16	0.16	0.16	0.24	0.16	0.08	0.00
26	NBPGR/TCR-1999	1.0	2.5	4.0	5.0	0.16	0.16	0.59	0.67	0.25	0.33	0.42	0.16	0.42	0.50	0.16	0.16
27	NBPGR/TCR-2042	1.0	2.8	3.6	4.5	0.14	0.22	0.29	0.31	0.06	0.00	0.32	0.31	0.29	0.26	0.06	0.00
28	NBPGR/TCR-1955	1.0	3.8	4.2	5.0	0.03	0.03	0.28	0.31	0.02	0.00	0.13	0.03	0.28	0.34	0.02	0.00
29	NBPGR/TCR-2040	1.0	2.5	2.9	4.5	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.46	0.14	0.16	0.63	0.00
30	NBPGR/TCR-2168	1.0	3.3	3.9	5.0	0.35	0.53	0.12	0.16	0.00	0.00	0.39	0.39	0.12	0.16	0.04	0.08
31	NBPGR/TCR-1988	1.0	2.1	2.9	4.4	0.10	0.07	0.02	0.03	0.00	0.00	0.35	0.27	0.12	0.10	0.04	0.04
32	NBPGR/TCR-1999	1.0	2.4	3.3	4.9	0.16	0.14	0.02	0.00	0.00	0.00	0.19	0.10	0.15	0.14	0.03	0.00
33	NBPGR/TCR-2146	1.1	2.6	3.4	4.5	0.11	0.21	0.02	0.05	0.03	0.03	0.21	0.25	0.13	0.16	0.33	0.45
34	NBPGR/TCR-2060	1.0	1.0	1.0	1.0	0.05	0.07	0.00	0.00	0.07	0.11	0.16	0.14	0.20	0.03	0.33	0.23
35	NBPGR/TCR-2055	1.0	2.9	3.6	4.8	0.16	0.20	0.05	0.05	0.03	0.05	0.25	0.16	0.09	0.04	0.16	0.04
36	NBPGR/TCR-2048	1.0	2.0	2.7	3.8	0.16	0.16	0.05	0.06	0.02	0.03	0.12	0.13	0.12	0.20	0.00	0.00
37	NBPGR/TCR-2019	1.0	2.0	2.5	5.0	0.00	0.00	0.08	0.00	0.00	0.00	0.08	0.16	0.00	0.00	0.00	0.00
38	NBPGR/TCR-1871	1.0	3.1	3.9	4.7	0.37	0.54	0.18	0.20	0.29	0.41	0.49	0.62	0.31	0.45	0.32	0.33
39	NBPGR/TCR-1783	1.0	2.0	2.3	3.9	0.12	0.24	0.08	0.16	0.08	0.16	0.31	0.31	0.08	0.08	0.25	0.17
40	NBPGR/TCR-1777	1.0	2.3	3.1	4.5	0.08	0.16	0.03	0.05	0.03	0.05	0.30	0.16	0.10	0.14	0.17	0.11
41	NBPGR/TCR-1552	1.0	3.3	4.0	5.0	0.00	0.00	0.04	0.00	0.16	0.31	0.12	0.16	0.08	0.08	0.16	0.24
42	NBPGR/TCR-808	1.0	1.3	1.3	1.3	0.24	0.34	0.06	0.09	0.46	0.34	0.11	0.03	0.05	0.05	0.57	0.28
43	NBPGR/TCR-1957	1.0	1.5	1.9	2.6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.31	0.75	1.50
44	NBPGR/TCR-776	1.0	2.0	2.6	3.9	0.12	0.16	0.00	0.00	0.04	0.00	0.21	0.16	0.08	0.08	0.47	0.70
45	NBPGR/TCR-2235	1.0	2.0	3.5	5.0	0.08	0.11	0.03	0.05	0.03	0.00	0.03	0.00	0.08	0.11	0.22	0.33
46	NBPGR/TCR-760	1.0	3.0	3.9	4.8	0.15	0.16	0.03	0.03	0.00	0.00	0.28	0.20	0.15	0.17	0.05	0.10
47	NBPGR/TCR-128-A	1.1	2.7	4.0	5.0	0.47	0.65	0.16	0.31	0.00	0.00	0.58	0.71	0.19	0.22	0.03	0.05
48	NBPGR/TCR-2137	1.0	2.0	3.5	5.0	0.24	0.31	0.33	0.50	0.24	0.31	0.24	0.31	0.16	0.16	0.24	0.31
49	NBPGR/TCR-2177	1.0	3.5	4.3	5.0	0.19	0.22	0.14	0.28	0.00	0.00	0.45	0.52	0.11	0.22	0.08	0.16
50	NBPGR/TCR-2173	1.2	3.5	4.2	5.0	0.04	0.08	0.00	0.00	0.08	0.16	0.16	0.16	0.04	0.00	0.12	0.24

51	NBPGR /TCR-2228	1.0	3.0	1.9	5.0	0.02	0.04	0.00	0.00	0.02	0.04	0.10	0.04	0.06	0.12	0.00	0.00
52	NBPGR /TCR-2192	1.0	1.5	3.5	5.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
53	NBPGR /TCR-2187	1.0	1.3	2.5	4.3	0.11	0.22	0.00	0.00	0.11	0.16	0.33	0.65	0.14	0.22	0.16	0.21
54	NBPGR /TCR-1753	1.0	3.0	3.3	4.5	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.31	0.08	0.16	0.08	0.00
55	NBPGR /TCR-1899	1.0	4.0	4.0	5.0	0.08	0.16	0.16	0.16	0.00	0.00	0.41	0.50	0.25	0.50	0.08	0.16
56	NBPGR /TCR-2061	1.0	2.7	4.2	5.0	0.11	0.21	0.00	0.00	0.16	0.31	0.16	0.11	0.05	0.05	0.24	0.31
57	NBPGR /TCR-2048	1.0	2.5	3.2	4.5	0.04	0.08	0.00	0.00	0.00	0.00	0.08	0.08	0.12	0.08	0.29	0.33
58	NBPGR /TCR-2235	1.0	3.0	3.3	4.2	0.23	0.46	0.00	0.00	0.00	0.00	0.24	0.31	0.16	0.31	0.00	0.00
59	NBPGR /TCR-1966	1.0	2.3	2.7	4.0	0.29	0.41	0.08	0.16	0.12	0.08	0.00	0.00	0.12	0.08	0.16	0.16
60	NBPGR /TCR-1975	1.0	1.5	2.5	4.2	0.31	0.46	0.16	0.31	0.08	0.00	0.80	0.87	0.60	0.73	0.84	1.37
61	NBPGR /TCR-1956	1.0	3.0	3.0	4.7	0.04	0.08	0.08	0.16	0.21	0.25	0.04	0.08	0.25	0.25	0.46	0.41
62	NBPGR /TCR-1934	1.0	5.0	5.0	5.0	0.08	0.16	0.16	0.16	0.16	0.16	0.40	0.80	0.17	0.00	0.16	0.16
63	NBPGR /TCR-1904	1.0	1.0	4.0	5.0	0.00	0.00	0.00	0.00	0.24	0.31	0.00	0.00	0.00	0.00	0.33	0.50
64	NBPGR /TCR-1479	1.0	1.5	3.5	5.0	0.00	0.00	0.00	0.00	0.24	0.31	0.00	0.00	0.00	0.00	0.08	0.16
65	Peechi Local	1.0	2.1	2.2	3.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.16	0.00	0.00
66	Kanhangad Local	1.0	1.0	1.5	1.9	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.16	0.17	0.33	0.30	0.00
67	Chittarikkal Local	1.0	2.5	3.7	4.9	0.25	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.16	0.00	0.00
68	Mavungal Local	1.1	3.3	4.4	5.0	0.08	0.16	0.00	0.00	0.00	0.00	0.16	0.16	0.08	0.16	0.08	0.16
69	Eranakulam Local	1.0	1.0	1.5	1.9	0.04	0.08	0.04	0.08	0.00	0.00	0.00	0.00	0.04	0.08	0.08	0.16
70	Mananthavady Local	1.0	2.9	3.3	3.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
71	Pudukad Local	1.0	1.0	1.0	1.1	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.04
72	Kollam Local-1	1.0	1.0	1.0	1.2	0.02	0.00	0.12	0.03	0.18	0.16	0.33	0.16	0.18	0.16	0.26	0.10
73	Kollam Local-2	1.0	1.8	3.0	4.7	0.11	0.11	0.00	0.00	0.06	0.11	0.03	0.00	0.00	0.00	0.12	0.19
74	Kilikolloor Local	1.0	1.3	1.7	2.2	0.00	0.00	0.02	0.00	0.02	0.04	0.00	0.00	0.06	0.12	0.52	0.87
75	Kattayikkonam Local	1.0	2.3	2.4	2.6	0.00	0.00	0.00	0.00	0.08	0.16	0.00	0.00	0.33	0.41	0.32	0.33
76	Kazhakkoottam Local	1.0	2.3	3.5	4.4	0.11	0.22	0.02	0.03	0.08	0.16	0.07	0.07	0.06	0.07	0.09	0.12
77	Nedumangad Local	1.0	1.0	1.0	1.1	0.00	0.00	0.08	0.16	0.00	0.00	0.00	0.00	0.11	0.22	0.08	0.05
78	Goureesapattom Local	1.1	1.4	1.7	3.6	0.00	0.00	0.00	0.00	0.03	0.03	0.17	0.00	0.13	0.10	0.03	0.03
79	Kakkamoola Local	1.0	1.0	1.5	2.5	0.00	0.00	0.02	0.04	0.02	0.00	0.00	0.00	0.06	0.08	0.06	0.04
80	Arka Anamika	1.1	2.2	3.5	4.3	0.00	0.00	0.02	0.00	0.16	0.31	0.16	0.05	0.04	0.00	0.05	0.04
81	NBPGR/TCR-874	1.1	3.5	4.0	5.0	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.04	0.08	0.16	0.16
82	MDU-1	1.0	1.1	1.3	1.9	0.10	0.00	0.08	0.00	0.04	0.04	0.21	0.16	0.04	0.08	0.08	0.12
83	NBPGR/TCR-985	1.0	2.0	2.9	4.2	0.04	0.07	0.35	0.70	0.19	0.34	0.13	0.00	0.08	0.12	0.00	0.00
84	NBPGR/TCR-893	1.0	2.2	2.9	3.9	0.04	0.00	0.08	0.05	0.25	0.14	0.03	0.00	0.15	0.19	0.21	0.25
85	Parbhani Kranti	1.0	1.0	1.0	1.0	0.39	0.40	0.02	0.03	0.11	0.22	0.18	0.08	0.19	0.31	0.38	0.70
86	Varsha Uphar	1.0	1.0	1.0	1.0	0.00	0.00	0.33	0.16	0.23	0.00	0.00	0.00	0.50	0.67	0.39	0.31
87	Salkeerthi	1.1	2.8	4.3	4.8	0.08	0.08	0.00	0.00	0.12	0.08	0.24	0.24	0.28	0.31	0.53	0.46
88	Aruna	1.0	3.0	3.8	5.0	0.03	0.00	0.24	0.31	0.03	0.05	0.11	0.00	0.08	0.16	0.03	0.00
89	Arka Abhay	1.0	1.0	1.5	2.3	0.06	0.00	0.00	0.00	0.04	0.08	0.18	0.12	0.06	0.12	0.02	0.00
90	Selection-13	1.0	1.0	1.0	1.1	0.03	0.00	0.00	0.00	0.00	0.00	0.12	0.05	0.10	0.19	0.11	0.22
91	Selection-46	1.0	1.0	1.0	1.0	0.08	0.05	0.00	0.00	0.00	0.00	0.35	0.30	0.03	0.05	0.15	0.05
92	Anakkomban-I	1.0	1.1	1.5	2.1	0.06	0.11	0.00	0.00	0.00	0.00	0.16	0.05	0.05	0.05	0.11	0.22
93	Anakkomban-II	1.0	1.0	1.9	3.2	0.03	0.00	0.00	0.00	0.00	0.00	0.13	0.05	0.11	0.11	0.03	0.05
94	Kiran	1.0	2.1	3.4	5.0	0.11	0.05	0.00	0.00	0.06	0.00	0.17	0.00	0.09	0.00	0.00	0.00
95	Kannur Local Red	1.0	1.5	2.0	4.0	0.03	0.00	0.00	0.00	0.00	0.00	0.08	0.11	0.08	0.11	0.03	0.00
96	Nileshwaram Local	1.0	1.0	3.0	5.0	0.16	0.16	0.04	0.08	0.10	0.12	0.10	0.08	0.04	0.04	0.08	0.12
97	Pananchery Local	1.0	3.0	3.0	5.0	0.00	0.00	0.00	0.00	0.07	0.14	0.18	0.12	0.16	0.16	0.24	0.24
98	Kalavoor Local	1.0	1.5	3.0	4.5	0.08	0.00	0.00	0.00	0.08	0.16	0.27	0.11	0.14	0.14	0.33	0.16
99	Balussery Local	1.0	4.0	4.5	5.0	0.06	0.08	0.12	0.20	0.16	0.16	0.04	0.04	0.17	0.25	0.10	0.12
100	Koyilandy Local	1.0	4.0	3.9	5.0	0.07	0.05	0.03	0.05	0.11	0.16	0.04	0.00	0.12	0.19	0.28	0.19
101	Payyannur Local	1.0	2.5	5.0	5.0	0.12	0.16	0.18	0.22	0.09	0.10	0.28	0.28	0.31	0.36	0.06	0.12
	Mean	1.0	2.2	3.0	4.1	0.1	0.13	0.11	0.13	0.07	0.08	0.18	0.17	0.19	0.21	0.17	0.17
	SE	NS	0.7	0.6	0.6	-	-	0.15	0.19	-	-	-	-	0.19	0.24	-	-
	CD	NS	1.8	1.7	1.5	NS	NS	0.39	0.05	NS	NS	NS	NS	0.51	0.62	NS	NS

Table 3. ANOVA for vectors and YVM incidence

Vectors	Mean square				
	Genotype (df=100)	Time (df=1)	Interaction (df=100)	Error (df=202)	Pooled error (df = 302)
Whitefly					
30 DAS	0.045**	0.329**	0.017	0.033	0.028
50 DAS	0.081**	0.152	0.014	0.031	0.025
70 DAS	0.032**	0.071	0.015	0.024	0.021
Leaf hopper					
30 DAS	0.094	0.069	0.044	0.073	0.064
50 DAS	0.110**	0.339	0.020	0.046	0.038
70 DAS	0.121	0.000	0.082	0.109	0.100

** Significant at 1% level

ii) **Association of YVM incidence with vector population**

Correlation coefficients of YVM incidence with populations of whitefly and leaf hopper were estimated at various stages of the crop *viz*., 30 DAS, 50 DAS, 70 DAS and final harvest (Table 4). YVM incidence at the three stages (except at 30 DAS) was significantly and positively correlated with the morning (0.209, 0.224 and 0.196 respectively) and evening (0.236, 0.251 and 0.210 respectively) populations of whitefly at 30 DAS. YVM incidence during last harvest exhibited positively significant correlation with morning (0.208) and evening populations of whitefly during 50 DAS also.

Morning population of leaf hopper at 30 DAS had significant positive correlation with disease incidence at 50 DAS (0.276) and 70 DAS (0.230). Evening count of leaf hopper had influence on YVM incidence at 50 DAS (0.301), 70 DAS (0.278) and final harvest (0.257).

It was clear from the association analysis that morning and evening populations of both whitefly and leaf hopper at 30 DAS had significant influence on the development of YVM from 50 DAS to final harvest. This indicates the fact that feeding by the vectors during the initial stage of crop growth, especially at 30 DAS, leads to the incidence and development of YVM disease throughout the crop phase. Moreover, whitefly count (both morning and evening) during 50 DAS also influenced the disease expression during the final phase of the crop. Bhagat *et al.* (2001) also arrived at similar conclusion that YVM disease development occurred at its maximum during 35 – 45 DAS of the crop. As evident from the table, population of both vectors at 70 DAS and leaf hopper count also at 50 DAS had no correlation with the disease incidence.

Table 4. Association of YVM incidence with its vector population

Vectors and time	Correlation coefficients with YVM incidence			
	30 DAS	50 DAS	70 DAS	Final harvest
Whitefly				
30 DAS				
Morning	0.045	0.209*	0.224*	0.196*
Evening	-0.002	0.236*	0.251*	0.210*
50 DAS				
Morning	-0.111	0.162	0.121	0.208*
Evening	-0.113	0.146	0.121	0.210*
70 DAS				
Morning	-0.030	-0.088	-0.071	-0.080
Evening	-0.025	-0.084	-0.014	-0.021
Leaf hopper				
30 DAS				
Morning	0.050	0.276**	0.230*	0.187
Evening	0.021	0.301**	0.278**	0.257**
50 DAS				
Morning	-0.042	0.146	0.074	0.171
Evening	-0.045	0.133	0.068	0.153
70 DAS				
Morning	-0.002	-0.096	-0.118	-0.082
Evening	-0.007	-0.052	-0.054	-0.029

*Significant at 5% level ** Significant at 1% level

The degree of Okra mosaic virus (OKMV) and the population of its vectors *viz*., *Podagrica unifoma* (Jac.) and *Podagrica sjostedti* (Jac.) at different growth stages of okra plants was studied using a netted barrier method and it was suggested that protecting okra plants up to 28 days after germination reduced the spread of OKMV by checking both vectors (Fajinmi and Fajinmi, 2010).

However, no information could be traced out from the available literature, regarding the magnitude and influence of vector population during various stages of crop growth on YVM disease development. Hence this study is the first stepping stone in this arena of research.

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First report of the genus *Aprostocetus* Westwood (Hymenoptera: Eulophidae) from Vietnam with descriptions of two new species

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ABSTRACT: The genus *Aprostocetus* Westwood is reported for the first time from Vietnam with descriptions of two new species *viz A. modistrus* Narendran & Nikhil, sp. nov. and *A. petiolatus* Narendran & Minu, sp. nov.

Key words: Oriental Region, Chalcidoidea, taxonomy, *Aprostocetus* Westwood, two new species

INTRODUCTION

The genus *Aprostocetus* Westwood belongs to the Subfamily Tetrastichinae of the family Eulophidae. Westwood (1833) described the genus with *Aprostocetus caudatus* Westwood as the type species. This is an extremely large genus, cosmopolitan in distribution. So far 758 valid species are known from the world of which 117 species are from Oriental region (Noyes, 2014). This genus has not yet been reported from Vietnam (Noyes, 2014) and the two new species and the unidentified species in this paper are the first report of the genus *Aprostocetus* Westwood from that country.

Genus *Aprostocetus* can be diagnosed by the presence of complete MS, bilobed or bidentate clypeal margin, 3 or 4 antennal funicular segments (anelli 3 or 4, rarely 1-2), 1 row of adnotaular setae on mesoscutum (rarely 2 or 3 rows), 2 pairs of setae on scutellum (rarely 3 pairs in aberrant forms) always near to SMG than to SLG (Narendran, 2007).

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The hosts of the members of this genus mostly include insects inhabiting plant galls, such as Diptera: Cecidomyiidae, sometimes Hymenoptera: Cynipoidea; occasionally Coleoptera or Coccoidea; rarely gall-inhabiting Acari (Graham, 1987).

Since the holotype specimens of the new species described here are on loan from Biodiversity Centre, Leiden (RMNH), they will be returned to RMNH [At present they are in ZSIK].

ABBREVIATIONS USED

AOL= distance between anterior ocellus and posterior ocellus; CC= costal cell; F1 to F4= funicular segments 1 to 4; L= length; ML= median line; MS= malar sulcus; MV= marginal vein; OD= ocellar diameter; OOL= distance between eye and posterior ocellus; OVPS= Ovipositor sheath; PMV= post marginal vein; POL= distance between posterior ocelli; SLG= sublateral groove; SMG= submedian groove; SMV= submarginal vein; STV= stigmal vein; W= width.

Acronyms of depositories: ZSIK= Zoological Survey of India, Kozhikode. RMNH= Biodiversity centre, Leiden, The Netherlands.

Aprostocetus modistrus Narendran & Nikhil, sp.nov.
urn:lsid:zoobank.org:act:8F72DA50-0DB5-40E3-8556-8A4FF3B6E35D
(Figs. 1-7)

Description: Holotype : 1♀, Length (excluding ovipositor sheath) 1.75mm. Head and mesosoma dark brown with slight metallic greenish blue refringence except the following: scape and legs pale whitish yellow, remaining segments of antenna pale yellowish brown. Metasoma pale yellowish brown with ovipositor sheath brown, wings hyaline with veins hyaline brown, tegula brown.

Head: Width in anterior view 1.35x (42:31) its height; width in dorsal view 2.15x (43:20) its height; eyes separated from each other in anterior view by 0.96x (23:22) eye height; eyes 1.3x (23:18) as long as wide; lower margin of clypeus bilobed; mandibles tridentate; eyes 3.3x (23:7) as long as malar sulcus; POL 2x OOL (9:5); POL 2.3x AOL (9:4); OOL 1.3x OD (5:4); distance between toruli 1.3x (5:4) the diameter of one torulus; distance between a toruli and an eye equal to diameter of one torulus (4:4); antenna inserted above level of lower eye margin, scape not reaching level of vertex; antennal formula 11441; clava unsegmented (Fig. 2); scape 0.9x (20:13) as long as eye; pedicellus plus flagellum 2.2x width of mesosoma (77:35). Relative L: W of antennal segments: scape = 20:4; pedicel = 7:3; F1 = 16:3; F2 = 16:3; F3 = 12:3; F4 = 9:3; clava = 16:4.

Mesosoma: Mesosoma with weak reticulations (Fig. 5), 1.6x as long as broad (56:35); pronotum 2.3x as broad as long (30:13); mesoscutum with one pair of adnotaular setae on each side, ML weak; mid lobe of mesoscutum 1.3x as long as broad (29:22); scutellum 1.05x as wide as long

(18:17); SMG with two pairs of stout setae; enclosed space between SMG 3x as long as broad (17:6), distance between SMG equal to distance between SMG and SLG; dorsellum clearly visible in dorsal view, 3x as wide as long (12:4), as long as median length of propodeum (4:4); propodeum with a distinct median carina, 8x as broad as its median length (32:4); callus with two setae on each side; rim of spiracle separated from posterior margin of metanotum by its own diameter; spur of midtibia 0.4x (5:13) as long as length of metatarsus. Relative L: W of hind leg: coxa = 24:11, trochanter = 6:5, femur = 35:7, tibia = 45:5, tibial spur = 8, tarsals 1 to 4 = 13, 9: 9, 9. Forewing 2.31x as long as broad (153:66); SMV with two dorsal setae (Fig. 7); relative L of, CC = 22, SMV = 23, MV = 58, STV = 13.

Metasoma: Gaster sessile, 4x as long as broad (96:25); 1.7x as long as mesosoma (96:56) and 1.3x (96:74) as long as combined length of head and mesosoma in side view; 5x as long as dorsal visible part of ovipositor sheath (96:20); 4 cercal setae, one black and longer than others, remaining 3 yellow and shorter; T1 longer than other tergites (Fig. 6), relative L:W of gasteral tergites: T1 = 21:24; T2 = 10:25;T3 = 12:25, T4 = 12:21; T5 = 11:17; T6 = 9:13; T7 = 9:25.

Male: Unknown

Host: Unknown

Material examined: Holotype: 1 ♀, NORTH VIETNAM: Hoa Binh. Pa Co Kang Kia N. R., 1051m N 20^{0}44'28" E 104^{0}55'45", 11. 23.x.2009, Mal. Trap. 25, RMNH 09. Coll. C.V. Achterberg & R. de. Vries.

Paratypes: 2 ♀♀, NORTH VIETNAM: Hoa Binh. Pa Co Kang Kia N. R., 1323m N 20^{0}44'36" E 104^{0}53'45", 11. 23.x.2009, Mal. Trap. 13, RMNH 09. Coll. C.V. Achterberg & R. de. Vries.

Etymology: The species name is derived from an arbitrary combination of letters.

Remarks: This species comes near Aprostocetus harithus Narendran in the key to species by Narendran (2007). It resembles A. harithus in general body colour, tridentate mandible, SMG with two pairs of stout setae, dorsellum almost as long as median length of propodeum, callus with two setae and SMV with two dorsal setae but differs from it in having: 1) MS slightly bent (slope 70^0) (in A. harithus, MS straight), 2) Antennal formula 11443 (in A. harithus, antennal formula 11433), 3) space between SMG 3x as long as broad (in A. harithus, space between SMG 1.8x as long as broad), 4) propodeum 8x as broad as its median length (in A. harithus, propodeum 11x as broad as its median length), 5) forewing 2.31x as long as broad (in A. harithus, forewing 2.77x as long as broad), 6) gaster 1.7x as long as mesosoma (in A. harithus, gaster 2.08x as long as mesosoma) and 7) gaster 5x as long as dorsal visible part of ovipositor sheath (in A. harithus, gaster 7.5x as long as dorsal visible part of ovipositor sheath).

This species comes near *Aprostocetus collega* Ratzeburg of Graham's key to European species

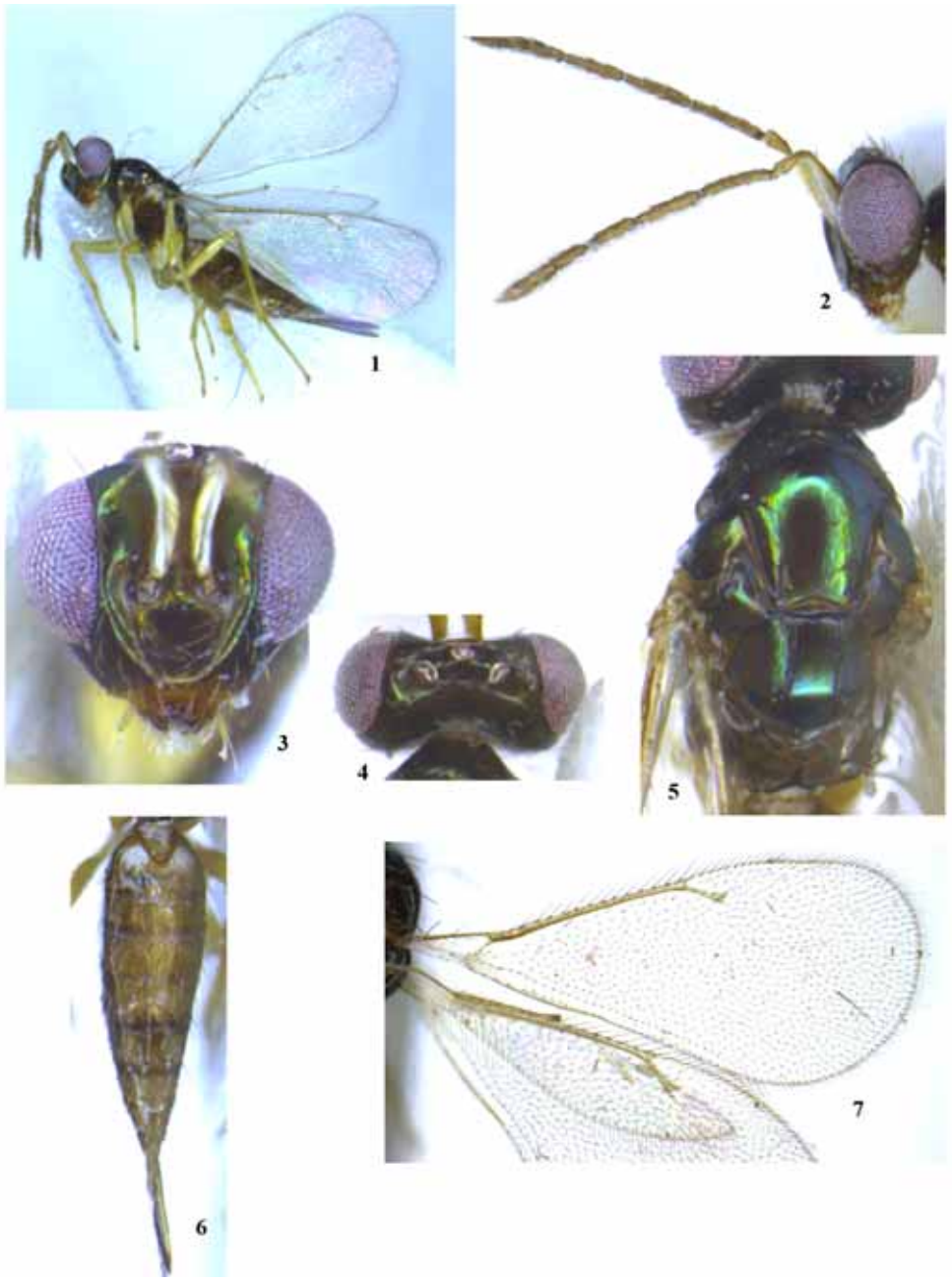
of *Aprostocetus* (1987). It resembles *A. collega* in having gaster 1.3x as long as head plus thorax but differs from it in having: 1) gaster 4x as long as broad (in *A. collega*, gaster 1.9-2.5x as long as broad) and 2) last tergite 3x as long as broad (in *A. collega*, last tergite 1.3x as long as broad).

Aprostocetus petiolatus Narendran & Minu, sp.nov.
urn:lsid:zoobank.org:act:B9A67AAA-1F23-41C6-B26B-0943515432EF
(Figs 8-15)

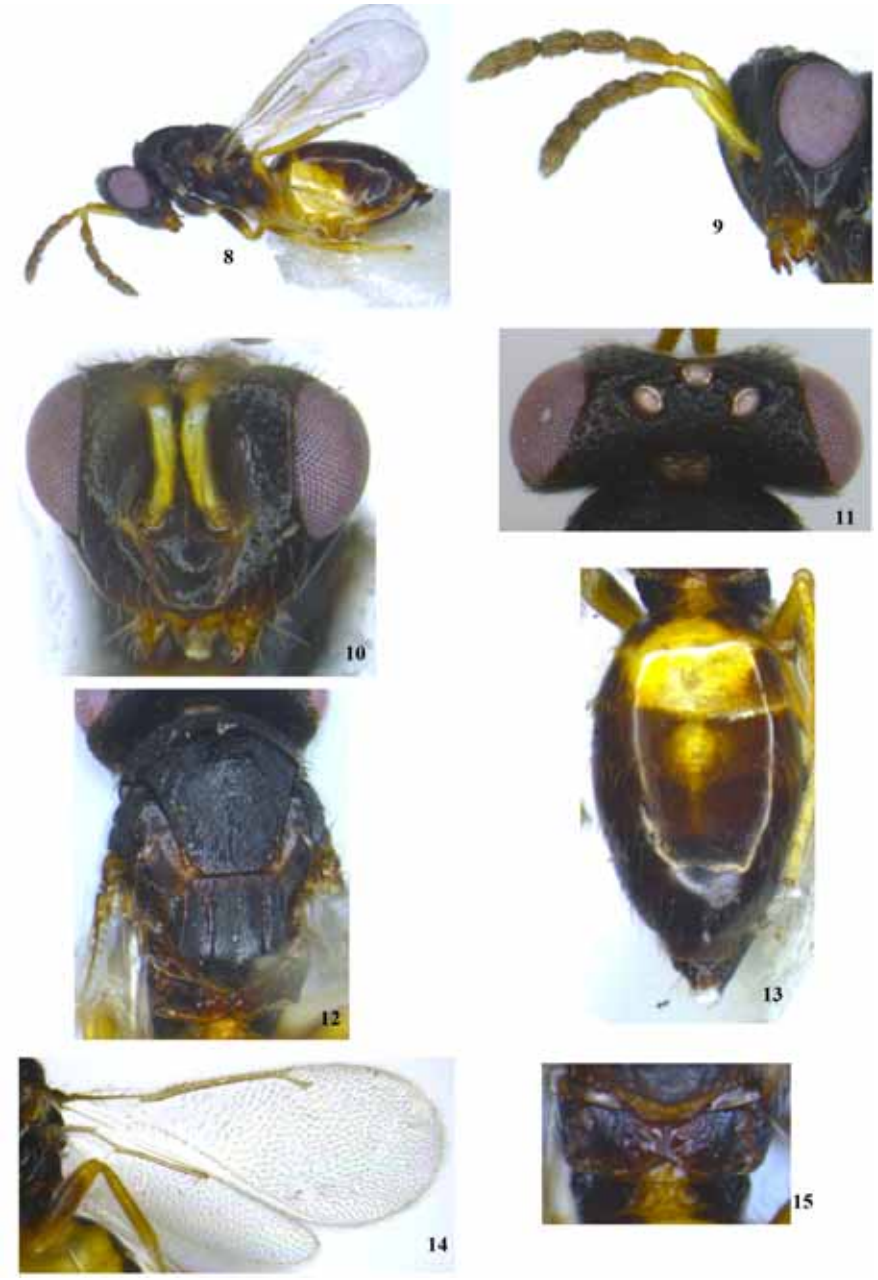
Description: Holotype : 1 ♀ Length 2.143mm. black with following parts as follows: scape, pedicel and anelli pale yellow; funicles and clava brownish yellow; mandibles pale golden yellow with brownish tinge at the tips; eyes grey; area adjacent to the longitudinal grooves of mesoscutum brown on either sides; dorsellum brownish yellow; propodeum brownish black; coxae concolorous with mesosoma except basal portion yellow; trochanters yellow; femur yellowish brown; remaining segments of legs yellow; wings hyaline with veins pale yellow; petiole golden yellow; T1 and T2 pale yellow with brownish patches on either sides; T3 brown with a pale yellow region in middle; T4 brown with black apex, remaining segments black.

Head: Width in anterior view 1.3x (64:49) its height; width in dorsal view 2.4x (68:28) its height; eyes 1.2x (30:25) long as broad and separated from each other in anterior view by 1.3x (39:30) eye height; area below torulus setose; lower margin of clypeus bilobed; mandibles bidentate; length of malar sulcus 0.43x (13:30) eye height; pedicel 0.3x (9:30) as long as eye height; pedicellus plus flagellum 0.75x (45:60) as long as width of mesosoma; distance between toruli 1.2x (7:6) diameter of one torulus; distance between a torulus and eye 1.8x (11:6) diameter of a torulus; POL 2.6x (13:5) AOL; POL 1.6x (13:8) OOL; OOL 1.3x (8:6) OD; antennae inserted above level of lower eye margin (fig.10); Antennal formula 11434; scape not reaching level of vertex, 0.77x as long as eye (23:30); Relative L:W of antennal segments: scape= 23:6, pedicel= 9:5,F1=12:7, F2=12:7, F3=2:7, clava=23:8. Funicles with sensillae and setae. Vertex sparsely setose (Fig.11).

Mesosoma: Mesosoma 1.32x (79:60) as long as wide; pronotum width 2.8x (50:18) its length; ML distinct; pronotum reticulate, sparsely setose (more than 20 setae) (fig. 12); Mesoscutum (excluding scapulae) 1.08x (40:37) as wide as its length, slightly less reticulate than pronotum; distal half of mesoscutum with notaular groove 0.62x (23:37) as long as mesoscutum; mesoscutum with 7 adnotaular setae, 5 near outer margin and 2 in the inner side; scutellum as wide as long (25:25); SMG 0.46x (6:13) nearer to SLG than to each other, space between SMG 1.85x (24:13) as long as broad; scutellum with a pair of proximal setae in middle region and a pair of distal setae near posterior margin; dorsellum 7x (21:3) as broad as long; propodeum 2.5x (45:18) as wide as long , 4x (45:12) as broad as its median length, with a strong median carina having a pair of setae arranged in a longitudinal row near lateral margin of spiracular grooves; callus with 3 setae; rim of spiracle fully exposed. Forewing 2.3x (149:65) as long as wide; SMV with 4 setae, MV with 14 setae on outer margin; speculum present; Relative length of CC= 35,



Figs. 1-7: *Aprostocetus modistrus* Narendran & Nikhil, sp.nov. (Female) 1. Profile, 2. Antenna, 3. Head anterior view, 4. Head dorsal view, 5. Mesosoma, 6. Gaster, 7. Wing



Figs. 8-15: ***Aprostocetus petiolatus*** **Narendran & Minu, sp. nov. (Female)**
8. Profile, 9. Antenna, 10. Head anterior view, 11. Head dorsal, 12. Mesosoma, 13. Gaster, 14. Wing, 15. Propodeum

SMV= 28, MV= 59, STV= 14; Relative L: W of hind leg: coxa= 29:10, trochanter= 8:6, femur= 46:11, tibia= 48:6, tibial spur= 9, tarsals 1 to 4= 9, 8; 8, 11.

Metasoma: Gaster 1.4x (110:79) longer than mesosoma, 1.13x (110:97) as long as combined length of head and mesosoma in side view; 1.9x as long as its width (110:57); Petiole 0.36x (5:14) as long as wide; T1, T2 and T3 smooth, remaining tergites faintly reticulate; Metasoma sparsely setose; 4 cercal setae, one black and longer than others, remaining 3 yellow and shorter; Relative L:W of gasteral tergites: T1= 17:41, T2= 16:55, T3= 20:59, T4= 20:53, T5= 19:45, T6= 6:25, OPSL= 8.

Male: Unknown

Host: Unknown

Material examined: Holotype: 1 ♀, NORTH VIETNAM: Hoa Tinh. Vu Quang N. P., 104m N $18^0 17' 45''$ E $105^0 25' 51''$, 24.ix - 5.x.2009, Mal. Trap. 19, RMNH 09. Coll. C.V. Achterberg & R. de. Vries.

Etymology: The species is named after its characteristic petiole.

Remarks: This species comes near *Aprostocetus malcis* Narendran in Narendran's key to species (2007) and resembles *A. malcis* in having similar head width in anterior view, median length of mesosoma and SMV with 4 dorsal setae, but it differs from *A. malcis* in having, 1) head width in dorsal view 2.4x as broad as long (in *A. malcis*, head width in dorsal view 3.3x as broad as long), 2) MS straight (in *A. malcis*, MS slightly bent), 3) scape not reaching level of vertex (in *A. malcis*, scape exceeding level of vertex, 4) dorsellum 0.2x as long as length of propodeum (in *A. malcis*, dorsellum 0.5x as long as length of propodeum), 5) propodeum 4x as broad as median length (in *A. malcis*, propodeum 5x as broad as median length) and 6) gaster petiolate (in *A. malcis*, gaster sessile).

This species also resembles *A. nadicus* Narendran in having straight MS, mesosoma 1.3x as long as broad, scutellum as long as broad and forewing 2.3x as long as broad but differs from *A. nadicus* in having, 1) space between SMG 1.85x as long as broad (in *A. nadicus,* space between SMG 2.75x as long as broad), 2) dorsellum 0.2x as long as median length of propodeum (in *A. nadicus*, dorsellum as long as length of propodeum), 3) propodeum 4x as broad as median length (in *A. nadicus*, propodeum 9x as broad as its median length) and 4) SMV with 4 dorsal setae (in *A. nadicus*, SMV with 6 dorsal setae).

A. petiolatus sp. nov. comes near *A. clavicornis* (Zetterstedt) of Graham's key to European species of *Aprostocetus* (1987). This species resembles *A. clavicornis* in general colour of scape and pedicellus, pedicellus about twice as long as broad, POL 1.5- 1.6x OOL, callus with 2- 4 setae, forewing 2.30- 2.35x as long as broad and SMV with 3- 5 dorsal setae but differs from it in having: 1) head twice as broad as mesoscutum (in A. clavicornis, head as broad as or

hardly broader than mesoscutum), 2) pronotum 0.5x as long as mesoscutum (in *A. clavicornis*, pronotum at most 0.25x as long as mesoscutum), 3) ML distinct (in *A. clavicornis*, ML usually absent) and 4) funicular segments equal in length (in *A. clavicornis*, funicular segments decreasing very slightly in length).

This species also resembles to *A. asperulus* (Graham) in having a straight malar sulcus, clava somewhat broader than funicle, callus with 3- 4 setae and forewing 2.3x as long as broad but differs from it in having, 1) thorax 1.3x as long as broad (in *A. asperulus*, thorax 1.5- 1.6x as long as broad), 2) pronotum 0.5x as long as mesoscutum (in A. asperulus, pronotum 0.2x as long as mesoscutum), 3) scutellum as long as broad (in A. asperulus, scutellum 1.2- 1.3x as long as broad), 4) dorsellum 7x as broad as long (in A. asperulus, dorsellum 4x as long as broad) and 5) gaster 1.9x as long as broad (in A. asperulus, gaster 3.5x as long as broad).

Aprostocetus sp.

One female, apparently of a new species, is left unnamed as the specimen is not in good condition. However, the same is treated as unidentified species and a description is given below.

Length (including OVPS) 3.87 mm.

Head: Width in anterior view 1.35x its height (54:40); width in dorsal view 2.07x its height (54:26); vertex with 4 stout spines on either side near eye margin; POL with 3 spines, one nearer to ocelli and one nearer to middle; one spine in front of posterior ocelli, nearer to AOL on either side; length of median spine 0.83x OD (5:6); eyes separated from each other in anterior view by 0.96x eye height (30:31); eyes 1.24x as long as wide (31:25); lower margin of clypeus bilobed; mandibles tridentate; eye 4.42x as long as malar sulcus (31:9); POL 1.6x OOL (11:7); POL 2.2x AOL (11:5); OOL 1.16x OD (7:6); distance between toruli 0.42x diameter of one torulus (6:7); distance between a torulus and eye 0.9x diameter of a torulus (6:7); antenna inserted 0.11mm above from lower margin of eye; Antennal formula 11442; scape 0.96x as long as eye (30:31); scape reaching above level of vertex; pedicellus plus flagellum 3.16x width of mesosoma (152:48); relative L:W of antennal segments: scape = 30:4; pedicel = 10:5; F1 = 45:4; F2 = 30:4; F3 = 27:4; F4 = 18:4; clava = 21:5.

Mesosoma: Mesosoma smooth, shiny with weak reticulations on pronotum and axillae, 2x as long as broad (91:48); pronotum weakly cross reticulated, flattened, 1.4x as broad as long (36:26); pronotum with 3 rows of setae, first row with 4 dorsal setae, second row with three pairs of setae and last row with 5 pairs of setae near ad margin; mid lobe of mesoscutum 1.06x as long as wide (33:31); 3 adnotaular setae on either side; ML absent; mesoscutum with posterior margin inverted 'V' shape medially; scutellum 1.13x as long as wide (27:24) with a pair of stout setae, one reaching middle and one near posterior margin; enclosed space between SMG 3.3x as long as wide (33:10); SMG slightly nearer to SLG than to each other;

dorsellum clearly visible in dorsal view, 0.5x as long as broad (9:19); 1.3x median length of propodeum (9:7); propodeum smooth, weakly reticulate than thorax with strong median carina; posterior margin developed as flaps on either side; propodeum 5.3x as broad as its median length (37:7); rim of spiracle exposed; callus with 3 setae; spur of mid tibia 0.3x as long as metatarsus (7:26); Relative L:W of hind leg, coxa = 39:18, trochanter = 13:6, femur = 65:12, tibia = 81:6, tarsals 1 to 4 = 31, 22: 15,12. Forewing 3x as long as broad (255:92); MV 6x STV; Relative L of, CC = 43, SMV = 45, MV = 107, STV = 17,

Metasoma: Sessile; 5.5x as long as broad (219:40); 2.4x as long as mesosoma (219:91) and 2x as long as combined length of head and mesosoma (219:129); 6.4x dorsal visible part of OVPS (219:34); all tergites smooth; Relative length of gasteral tergites, T1 = 38, T2 = 24, T3 = 25, T4 = 23, T5 = 28, T6 = 22, T7 = 65, OVPS (side view) = 103.

Material examined: 1 ♀, NORTH VIETNAM: Hoa Binh. Pa Co Kang Kia N. R., 1051m N 20^{0}44$'$28$''$ E 104^{0}55$'$45$''$, 11. 23.x.2009, Mal. Trap. 25, RMNH 09. Coll. C.V. Achterberg & R. de. Vries.

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Adulticidal and repellent activities of *Melaleuca leucadendron* (L.) and *Callistemon citrinus* (Curtis) against filarial and dengue vectors

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ABSTRACT: Essential oils extracted by steam distillation from two tropical plants viz; *Melaleuca leucadendron* (L.) and *Callistemon citrinus* (Curtis) evaluated against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say), *in vitro* for their adulticidal and repellent potentiality showed a 100% protection up to 5 hrs after treatments. *M. leucadendron* showed 100% repellency against *Cx. quinquefasciatus* and 80.9% repellency against *Ae. aegypti* at its 8th hour of exposure. Adulticidal activities of these two essential oils observed after 24 hour exposure showed 100% adult mortality, indicating their potentiality in the control of mosquitoes.

KEYWORDS: *Aedes aegypti, Culex quinquefasciatus, Melaleuca leucadendron, Callistemon citrinus,* essential oil, repellent activity, adulticidal activity.

INTRODUCTION

Mosquitoes are well known for public health importance, as they are the vectors which transmit pathogens of several diseases like malaria, chikun guniya, dengue fever, yellow fever, Japanese encephalitis and so on (Service, 1983). Mosquitoes alone transmit diseases affecting more than 700 million annually (Jang *et al.,* 2000). Man started using synthetic chemical insecticide DDT since 1940 to control mosquitoes (Metcalf and Lukaman, 1975). Indiscriminate use of synthetic insecticides lead to various environmental consequences (Agarwal *et al.,*1981), development of genetic resistance in mosquito species (Sharma *et al.,*1986) and disrupted natural biological control systems (Brown, 1986). Plant-derived

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essential oils can be considered as a valuable alternative for insect control (Govindarajan *et al*., 2008).

Repellents of plant origin should be non-toxic, non-irritating and long lasting and eco-friendly in nature. Plants of terrestrial origin have been reported to be an important source of mosquito repellents (Hwang *et al*., 1985). The essential oils from medicinal herbs in Lebanon proved as an environmentally safe measure to control the seaside mosquito (Knio *et al*.,, 2008). Several essential oils from herbs act as *Culex* and *Aedes* larvicides (Sukumar *et al.,* 1991). Essentials oils from *Cannabis sativa* (Thomas *et al.,* 2000) and *Tagetes patula* L. (Dharmaggada et al., 2005) were reported to have activity against *Aedes aegypti* Linn., *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say. Prajapati *et al.* (2005) studied the larvicidal, adulticidal, oviposition deterrent and repellent activities of essential oils from 10 medicinal plants against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The undiluted oils of *Cymbopogon nardus* Linn., *Pogostemon cablin* Benth., *Syzigium aromaticum* Linn. and *Zanthoxylum limonella* Alston were the most effective and provided two hours of complete repellency (Trongtokit *et al*., 2005).

MATERIALS AND METHODS

Target organism: Mosquito species chosen for the present study were *Ae.aegypti* and *Cx. quinquefasciatus* obtained from the laboratory culture maintained as described in Pushpalatha and Muthukrishnan (1995).

Essential Oil extraction: Leaves of *Melaleuca leucadendron* Linn. and *Callistemon citrinus* (Curtis) Skeels were collected from the field, washed with distilled water and essential oils were extracted by steam distillation in Clevenger-type apparatus (Craveiro *et al.,* 1976).

Repellency Assay: Repellency of volatile oils was evaluated using human bait technique. Each test was conducted for a period of 8hrs, depending on the response. *Ae. aegypti* was tested between 07.00 h and 15.00 h while *Cx. quinquefasciatus* was tested between 17.00 h and 01.00 h. Each oil was tested 100, 70, 50 and 10 percent concentrations. An arm of a human volunteer was covered with a paper sleeve with 3x10cm window and 0.1ml of desired concentrations of the oil was applied. The uncovered arm was exposed into a standard mosquito emergence cage having 100 hungry 4 to 5 days old female mosquitoes for one minute. Prior to the commencement of each exposure, the mosquitoes were tested for their readiness to bite by placing an untreated bare hand of a volunteer for 30 seconds. An arm of human volunteer without any oil application was kept as control. The number of incidence of landing without biting and those of biting ones were recorded at each interval until the rate of bite fell into 1to 1.5 per minute. The duration between the application of repellent and the commencement of bite was recorded as the protection time. The percentage of repellency was calculated at the end of every test using the formula mentioned by Tawastsin *et al* (2001) viz; (C-T/C) x 100 where, C is total number of mosquitoes landing or biting the control area and T is total number of mosquitoes landing or biting the treated area.

Adulticidal Bioassay: Sugar fed adult mosquitoes (4-6 days old) was used for bioassay. Different concentrations of the essential oils were impregnated on filter papers of 1cm^2 size. The bioassay was conducted in a cylindrical glass tube (15cm X 5cm) following the method of WHO (1981). The experiment was carried out in triplicate for each essential oil. For each replicate two tubes were used; one was used to expose the mosquitoes to the essential oil and another to hold the mosquitoes before and after the exposure period. Each tube was closed at one end with a wire mesh screen. Twenty sucrose fed mosquitoes were released in to the tube, and the mortality rate was observed every 15 min for 3h exposure. At the end of the exposure period, the mosquitoes were transferred in to the holding tube. A cotton pad soaked in 10% sugar solution was placed in the tube during the holding period. Mortality of mosquitoes recorded after 24h.

RESULTS AND DISCUSSION

Observations of the present study showed that the essential oils of *M. leucadendron* and *C. citrinus* have significant adulticidal and repellent activity against *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes. The present findings are comparable to earlier reports of Amer and Mehlhorn (2006), who reported forty one essential oils against different species of mosquitoes

Table 1. Evaluation of different concentrations of essential oils of *M. leucadendron* and *C. citrinus* against selected species of mosquitoes

Plants	Mosquito species	Conc. (%)	Percentage of repellency at different intervals after treatment					
			330 min	360 min	390 min	420 min	450 min	480 min
M. lecadendron	*Cx. quinquefasciatus*	100	100	100	100	100	100	100
		50	100	100	100	100	100	100
		10	100	100	100	100	96	84
		0	0	0	0	0	0	0
	Ae. aegypti	100	100	100	100	100	97	90
		50	100	100	100	96.5	88	80.9
		10	100	94	87	82	75	70
		0	0	0	0	0	0	0
C. citrinus	*Cx. quinquefasciatus*	100	100	100	100	100	100	97
		50	100	100	100	100	94.9	85.7
		10	100	100	94.2	88.7	83	79
		0	0	0	0	0	0	0
	Ae. aegypti	100	100	100	100	100	96	88.5
		50	100	100	94	88.2	81	75.7
		10	94.2	89	80	76	70.3	66
		0	0	0	0	0	0	0

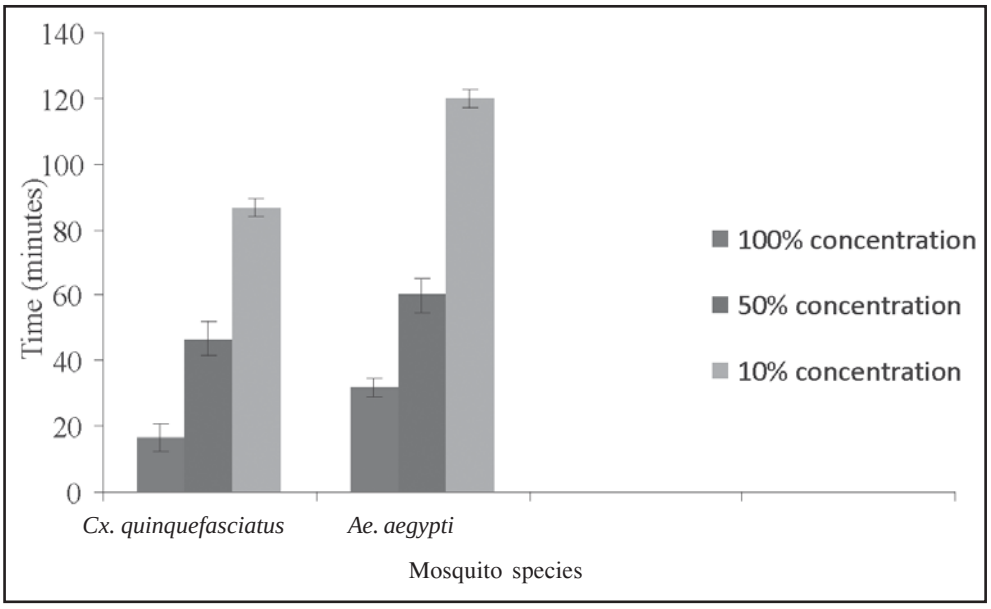

Figure 1. Time (minutes) taken for 100% adult mortality of mosquitoes tested with different concentration of *M. leucadendron* essential oil for an exposure time of 3 hours

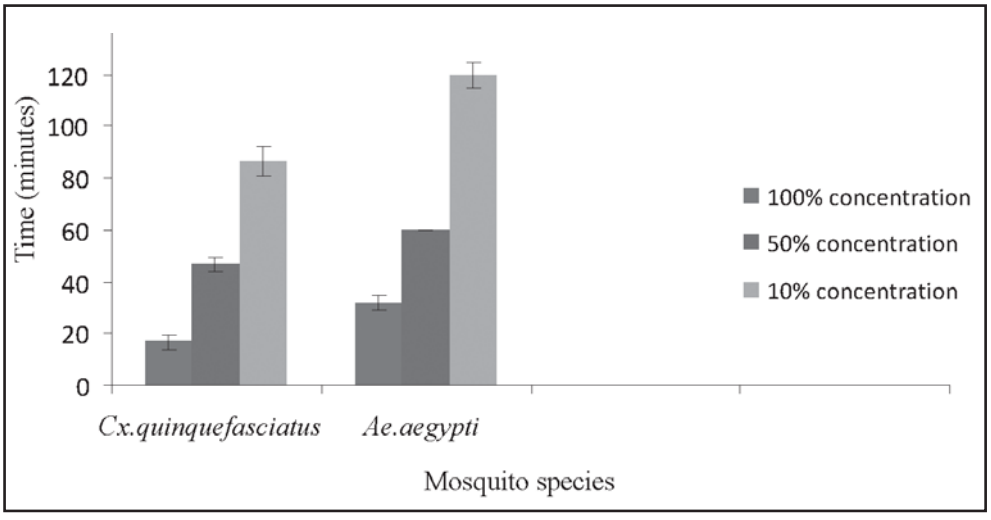

Figure 2. Time (minutes) taken for 100 % adult mortality of mosquitoes tested with different concentration of *C. citrinus* essential oil for an exposure time of 3 hours

and found out five most effective essential oils viz; *Litsea cubeba* (Lour.) Pers., *M. leucadendron, M. quinquenervia* (Cav.) S. T. Blake, *Viola odorata* Linn. and *Nepeta cataria* Linn., which induced 100% repellency over a protection period of 480 min against *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus.* Observations on the efficacy of essential oils, the repellent effect and adulticidal effect against *Cx. quinquefasciatus* and *Ae. aegypti* are provided in table 1.

Considering protection time and repellency, *M. leucadendron* was shown to have higher activity than that of *C. citrinus*. The percentage repellency showed a dose depended effect on the tested mosquitoes. In *Cx. quinquefasciatus*, the percentage repellency of *M. leucadendron* ranged between 84% - 100%. The 100% protection period lasted up to 480 min for 100% and 50% concentrations. At 10% concentrations the 100% protection period lasted up to 420 min. *M. leucadendron* demonstrated 420 min protection period against *Ae. aegypti.* In case of *C. citrinus* essential oil, the protection period for both *Cx. quinquefasciatus* and *Ae. aegypti* were 420 min. The percentage repellency for 480 min at 100%, 50% and 10% concentrations were 97%, 85.7% and 79% for *Cx. quinquefasciatus* and 88.5%, 75.7% and 66% for *Ae. aegypti* respectively.

Observations on adulticidal activity of *M. leucadendron* and *C. citrinus* were provided in figure 1 and 2. Among the two essential oils tested, the highest adulticidal activity was observed in *C. citrinus* against *Cx. quinquefasciatus*. At higher concentrations, the adults showed the restless movement for sometimes with abnormal wagging and died. The results show a dose depended effect on adult mortality. From these results, it is proved that both *M. leucadendron* and *C. citrinus* are active agents against mosquitoes. This study opens the possibility of further investigations of these plant products against other pest populations. Further studies are needed to develop appropriate formulations. The isolation and purification studies are in progress.

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Effect of spinosad 45 SC on growth and Development of Entomopathogenic Fungi *Metarhizium anisopliae* and *Beauveria bassiana*

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ABSTRACT: An experiment was conducted to assess the *in vitro* effect of spinosad 45 SC (Tracer ®) on the entomopathogenic fungi *viz.*, *M. anisopliae* and *B. bassiana.* The insecticide was applied at various concentrations and the field dose and higher doses adversely affected colony development, sporulation and spore germination. The effect was significantly higher on *B. bassiana* than on *M. anisopliae* and the effect increased with the dosage of insecticide used.

Key words: Spinosad 455C, entomopathogenic fungi, sporulation, inhibition.

INTRODUCTION

Concerns in the use of entomopathogenic fungi as alternative pest control agents are increasing even where chemical pesticides have been used as the main agents for controlling pests and diseases in crop production (Jeong Jun and Kyu Chin, 2007). The entomopathogenic fungus, *Metarhizium anisopliae* and *Beauveria bassiana* are the facultative insect pathogens with significant host range and host specificity. They are registered as biopesticides with a broad host range and used for management of several insect pests (Amutha *et al.,* 2010). The insecticide and entomopathogenic fungi are often used in combination for managing insect

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pests of crops, especially vegetables due to their low toxicity and low residual effect. Such use of microbial pesticides combined with pesticides in pest management practices requires detailed compatibility study. The use of incompatible insecticides may inhibit the development and reproduction of the pathogens, affecting the result in IPM practices. Keeping this in view, present investigation was taken up to study the effect of spinosad 45 SC on growth and development of *M. anisopliae* and *B. bassiana* under *in vitro* condition.

MATERIALS AND METHODS

The study was conducted at the Kerala Agricultural University, College of Agriculture, Padanakkad, Kasaragod, Kerala, India. Poisoned food technique (Nene and Thapliyal, 1997) was adopted for the experiment. Radial growth of the test isolates were recorded 10, 12 and 14 days after inoculation. Per cent inhibition of growth over control was calculated using the formula (Vincent, 1927), I=C-T/C×100. Where I = per cent inhibition, C = growth of micro organism in untreated medium and T = growth of micro organism in treated medium.

After *in vitro* effect of spinosad on *M. anisopliae* and *B. bassiana*, sporulation assay was carried out using Martins' rose Bengal agar media. The media was prepared using 5 g peptone, 10 g dextrose, 20 g agar, one gram KH_2PO_4, 0.5 g $MgSO_4.H_2O$, 30 mg Rose Bengal and one litre distilled water. All the ingredients except agar were dissolved in 500 ml distilled water while 20 g agar was dissolved in 500 ml distilled water by boiling. They were then mixed thoroughly and to this mixture Rose Bengal dye was added. This was then poured in to two 500 ml conical flasks and plugged with cotton and sterilized in autoclave at 121.0 ^{0}C for 15 to 20 minutes.

The spore suspension of entomopathogenic fungi were prepared by serial dilution, using *M. anisopliae* and *B. bassiana* growth in different treatments. One milliliter spore suspension of the test fungus was poured in a sterilized petri plate. Thereafter, 20 ml molten and cooled Rose Bengal agar medium was poured in to the petri plate containing spore suspension under aseptic condition and was allowed to solidify and was incubated at 28±1^{0}C in an incubator. Number of colonies of the test isolates was recorded after 10 to 14 days of incubation. The spore germination inhibition was assessed following the method of Peterson (1941). The number of germinated spores in the field was recorded 12 hr after incubation under a compound microscope (40 X objective). From each replication, five microscopic fields were observed following the method of Yashoda (1998), for converting average number of spores germinated per microscopic field.

Per cent spore germination inhibition was calculated using the formula developed by Verma and Singh (1987), I=C-T/C×100. Where I = per cent inhibition, C = number of spores germinated in control and T = number of spores germinated in treatment.

RESULTS AND DISCUSSION

Minimum percent of inhibition (5.23) of colony diameter of *M. anisopliae* was found in samples

treated with 0.0018 % (recommended concentration) concentration of spinosad 45 SC at 14 days after inoculation, while the maximum (26.75) per cent inhibition was found in samples treated with 0.0054 % of spinosad 45 SC. The spinosad 45 SC at the concentration of 0.0036 % recorded 10.44 per cent inhibition of colony diameter of *M. anisopliae.* The present findings were not in agreement with the earlier report of Asi *et al.* (2010) that, spinosad 45 SC was safe to conidial germination and growth of *M. anisopliae*. The present study revealed that, spinosad 45 SC inhibits *M. anisopliae* colony and hence it is not safe for conidial germination (Table 1).

The lowest (23.22) per cent of inhibition of colony diameter of *B. bassiana* was found in sample treated with 0.0018 % concentration of spinosad 45 SC at 14 days after inoculation and it was on par with 0.0036 % concentration with 29.62 per cent inhibition. The highest (47.76)

Table 1. Effect of spinosad 45 SC on growth of *M. anisopliae* in *in vitro* culture

Treatments	Inhibition of colony diameter of *M. anisopliae* (cm)*			Per cent inhibition of colony diameter of *M. anisopliae* *		
	10 DAI	12 DAI	14 DAI	10 DAI	12 DAI	14 DAI
T_1 : Spinosad 45 SC @ 0.0018%	3.35	3.47	4.07	4.24	4.73	5.23
T_2 : Spinosad 45 SC @ 0.0036%	3.27	3.32	3.85	6.41	8.86	10.44
T_3 : Spinosad 45 SC @ 0.0054%	2.65	2.72	3.15	24.22	25.20	26.75
T_4 : Control	3.50	3.65	4.30	-	-	-
SE @0.05 %	0.13	0.14	0.13	1.03	1.79	0.81
CD @0.05 %	0.04	0.04	0.04	3.31	5.79	2.61

*Mean of four replications DAI- Days After Inoculation

per cent inhibition of colony diameter was found in samples treated with 0.0054 % concentration at 14 days after inoculation. The present finding is not in agreement with Amutha *et al.* (2010) who reported that, spinosad 45 SC is slightly toxic to *B. bassiana.* The present study revealed that there was drastic reduction of colony growth of *B. bassiana* when treated with recommended dosage of spinosad 45 SC (Table 2).

All the tested concentration of spinosad 45 SC gave 100 per cent inhibition of sporulation and spore germination on *B. bassiana,* but in the case of *M. anisopliae,* minimum (3.76 per cent) inhibition of sporulation and (26.82 per cent) spore germination was found in the 0.0018 % concentration and highest inhibition of (17.86 per cent) sporulation and (82.73 per cent) spore germination was recorded in samples with 0.0054 % concentration. The present findings were not in agreement with the earlier report of Akbar *et al.* (2012) where they reported that spinosad was compatible with *M. anisopliae* and was found safe to conidial germination and growth of the fungi. The result of present study shows that, the spinosad inhibits conidial germination

Table 2. Effect of spinosad 45 SC on growth and development of B. bassiana

Treatments	Inhibition of colony diameter of *B. bassiana* (cm)*			Per cent inhibition of colony diameter of *B. bassiana* *		
	10 DAI	12 DAI	14 DAI	10 DAI	12 DAI	14 DAI
T1 : Spinosad 45 SC @ 0.0018%	2.00	2.55	2.95	8.82	20.20	23.22
T2 : Spinosad 45 SC @ 0.0036%	1.82	2.35	2.70	16.79	26.36	29.62
T3 : Spinosad 45 SC @ 0.0054%	1.67	1.72	2.00	23.65	46.03	47.76
T4 : Control	2.20	3.20	3.85	-	-	-
SE @0.05 %	0.15	0.14	0.25	2.39	1.82	2.72
CD @0.05 %	0.04	0.04	0.08	7.65	5.82	8.71

*Mean of four replications DAI- Days After Inoculation

and growth of *M. anisopliae* and hence it is not safer to the fungi. The spore germination of *B. bassiana* was totally inhibited by the spinosad and hence the current studies are not in accordance with the results of Rajanikanth *et al.* (2010) who reported that *B. bassiana* is compatible with spinosad (Table 3 and 4).

From the study, it was clear that both the fungi tested are not compatible with the spinosad 45 SC in the laboratory condition. This need to be ascertained under field condition, since, the field condition may have lower dose of insecticide due to drift and further break down of insecticide.

It is inferred that the insecticide spinosad 45 SC inhibits the growth of both the pathogens. The adverse effect is higher in *B. bassiana* than on *M. anisopliae*. The adverse effect is

Table 3. Effect of spinosad 45 SC on sporulation of entomopathogenic fungi

Treatments	Mean count of colony *		Inhibition of sporulation (%)*	
	M. anisopliae	*B. bassiana*	*M. anisopliae*	*B. bassiana*
T_1 : Spinosad 45 SC @ 0.0018%	312.50	0.00	3.76	100.00
T_2 : Spinosad 45 SC @ 0.0036%	290.00	0.00	10.69	100.00
T_3 : Spinosad 45 SC @ 0.0054%	266.75	0.00	17.86	100.00
T_4 : Control	324.75	0.00	-	-
SE @0.05 %	3.83	-	0.36	-
CD @0.05 %	1.24	-	1.16	-

*Mean of four replications

Table 4. Effect of spinosad 45 SC on spore germination of entomopathogenic fungi

Treatments	*M. anisopliae*		*B. bassiana*	
	Germinated spores (%)	Inhibition of spore germination (%)*	Germinated spores (%)	Inhibition of spore germination (%)*
T1 : Spinosad 45 SC @ 0.0018%	11.24	26.82	00.00	100.00
T2 : Spinosad 45 SC @ 0.0036%	36.05	44.65	00.00	100.00
T3 : Spinosad 45 SC @ 0.0054%	47.65	82.73	00.00	100.00
T4 : Control	65.13	-	30.50	-
SE @0.05 %	0.45	0.81	-	-
CD @0.05 %	1.41	2.49	-	-

*Mean of four replications

lowest in field dose of the insecticide and increases with higher doses. With *M. anisopliae* the growth, sporulation and spore germination are significantly lower than in control but the differences are not very high. Since the response is seen as dose dependent by lowering the dose in field use it may be possible to nullify the adverse effect of combining treatments. Regarding *B. bassiana* which shows high growth suppression and 100 per cent suppression on sporulation and spore germination even at field level of the insecticide, a combined use may not be advantageous.

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Analysis of the boil-off loss in parental and different crosses of bivoltine silkworm, *Bombyx mori* L.

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ABSTRACT: The process of removal of gummy proteinous material (sericin) from the silk is commonly referred to as degumming loss or boil-off loss and is considered as one of the important economic traits during the course of silkworm breeding. Loss of sericin varies with breeds / hybrids, it is essential to analyze the ratio with reference to cocoon shell, as it is the basic raw material for the raw silk to estimate silk productivity of a breed / hybrid. In the present study, boil-off loss ratio in the cocoon shells of twelve bivoltine parental breeds (6 oval and 6 dumb-bell) and newly developed 21 each of different crosses (single, three-way and four-way crosses) of bivoltine hybrids along with control hybrids CSR2xCSR4 and [(CSR2xCSR27)x(CSR6xCSR26)] was analyzed to identify promising hybrids with desired boil-off loss ratio. Heterosis for boil-off loss ratio, shell ratio and raw silk percentage both in parents and all the hybrid crosses was also estimated and discussed. Among the parents, the least value of 21.50% was registered in JPN8 (oval), 22.01 % (S9) amongst dumb-bells and in the hybrids, single-cross hybrid CSR27xCSR26 (23.25%), three-way cross hybrid, (JPN8xCSR17)xCSR26 (22.51%) and four-way cross hybrid [(JPN8xCSR17)x(D13xCSR26)] (22.33%) was recorded. It was observed that some of the different crosses of the hybrids have also recorded desirable heterosis values for boil-off loss trait.

KEYWORDS: Bivoltine silkworm, *Bombyx mori* L., Sericin, Boil-off loss, economic trait, heterosis, single, three-way and four-way cross.

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INTRODUCTION

To meet the requirement of the consumers across the world, sericulture industry requires superior quality of silk to produce internationally gradable silk fabric and increased foreign exchange. To face the global competitiveness in silk production there is a need to improve the quality of the raw silk. At the end of larval period, the silkworm spins a shell by extruding silk bave for protection during its pupation from adverse environment.

The silk bave composed of two protein substances viz., fibroin and sericin also contains a small quantity of fatty, waxy, coloring and mineral matters. Fibroin, axis of silk thread, represents 70-80% of its weight, whereas, sericin, encloses fibroin in a common sheet, accounts for 20-30% of the weight. The fatty, waxy, coloring and mineral matter forms very meager part (2-3%) of the silk bave.

Degumming is the process of removal of sericin in boiling soap solution and the cocoon shell has more boil-ff loss ratio when compared to the raw silk (Kannan, 1986). The main silk substance, fibroin is insoluble in alkaline hot water, whereas, sericin (silk gum) is easily soluble in boiling alkaline soap solution (Sadov et al., 1978). Boil-off loss ratio, one of the important qualitative traits during breeding, for bivoltine race is found to be 24% (optimum) and it is genetically different among silkworm strains (Sinha et al., 1992). The boil-off loss ratio is comparatively higher in polyvoltines than bivoltines because of more floss (Sidhu and Sonwalker, 1969, Naseema Begum et al., 2010). Low boil-off loss ratio improves cocoon reeling qualities (Gamo and Hirabayashi, 1984). The boil-off loss varies according to the seasons and is influenced by environment (Sonwalker, 1969).

As the loss of sericin and its effect on weaving and other post weaving processes due to degumming, varies with different breeds, it is necessary to study in detail the boil-off loss with reference to cocoon shell itself, as it is the basic raw material for the raw silk. In light of the above, the present study was undertaken to analyze the boil-off loss in bivoltine parental breeds and their different cross hybrids to identify and select the promising hybrids.

MATERIALS AND METHOD

Six each of oval and dumb-bell bivoltine silkworm breeds viz., CSR17, CSR27, JPN8, JPN7, S5, BBE226 (oval) and CSR16, CSR26, D13, S9, BBE247, BBE247 (dumb-bell) were utilized for preparation of single, three-way (oval x oval) x dumb-bell and four-way cross (oval x oval) x (dumb-bell x dumb-bell) hybrids by employing partial diallel-cross technique. The parental breeds and hybrids were brushed together and reared in three replications by the standard rearing technique (Datta, 1992) during different seasons of the year viz., summer, rainy and winter.

After the sex-separation and assessment, sixty cocoon shells (thirty each of female and male) were taken at random and were replicated into three, with ten each of male and female, for

conducting the experiment. Degumming was carried out by boiling the cocoon shells in standard soap solution (Basavaraja et al., 2000).

The method followed for degumming of the cocoon shells is by using neutral liquid soap for boiling. Degumming of raw silk was carried out by using the material to liquor ratio of 1: 40 prepared by dissolving a quantity of 5g / liter neutral soap with 1g / liter sodium carbonate (soda) and was boiled in a copper vessel at a temperature of 90 to 95ºC for 45 minutes. After a shorter time gap, the sample material was rinsed in hot water for 30 minutes and then in cold water before being hydro extracted. The degummed cocoon shells were then transferred to perforated paper envelops and dried in oven maintaining temperature of 105ºC for 5 hours. The initial dry weight and final dry weight of the degummed silk were considered for calculation of boil-off loss by following the formula,

$$\text{Boil-off Loss Ratio (\%)} = \frac{\text{Initial dry weight} - \text{final dry weight after degumming}}{\text{Initial dry weight}} \times 100$$

The hybrid vigour of different crosses was estimated by using the following formula,

$$\text{Heterosis (\%)} = \frac{\text{Hybrid value} - \text{Mid parent value (MPV)}}{\text{Mid parent value (MPV)}} \times 100$$

Multiple trait evaluation index (Mano et al., 1993) was estimated by using the following formula:

$$\text{Evaluation Index (E. I.)} = \frac{(A - B)}{C} \times 10 + 50$$

Where, A = Value of a particular breed for particular trait,

B = Mean value for a particular trait of all the breeds,

C = Standard Deviation of a particular trait for all the breeds,

10 = Standard unit,

50 = Fixed value.

Minimum/average E.I. value fixed for selection of a breed is >50.

Cocoons selected randomly were cut, sex separated at pupal stage and weighed for the cocoon weight and shell weight. The average weight of 20 (10 male and 10 female) shells was taken as cocoon weight and shell weight. Shell ratio was estimated by following the formula,

$$\text{Shell ratio (\%)} = \frac{\text{Weight of cocoon shell}}{\text{Weight of cocoon}} \times 100$$

Raw silk percentage, defined as the quantity of raw silk obtained after reeling 100 kg of cocoons, was calculated by using the following formula,

$$\text{Raw silk (\%)} = \frac{\text{Weight of raw silk reeled + converted silk weight of carry over cocoons}}{\text{Weight of cocoons taken for reeling}} \times 100$$

RESULTS

The mean values of performance showing shell ratio, raw silk percentage and boil-off loss in oval and dumb-bell bivoltine parental breeds, exhibiting variations for these traits, is detailed in Table-1. Among oval breeds, shell ratio ranged from 23.33% (BBE226) to 25.01% (CSR27), raw silk percentage from 17.09% (BBE226) to 18.40% in CSR27 and in boil-off loss, JPN8 (21.50%) and JPN7 (21.90%) lines registered lower values against the average of 22.78%. Whereas, in dumb-bell breeds shell ratio ranged from 18.15% (BBE267) to 22.18% (CSR16), raw silk percentage from 16.30% (BBE267) to 19.22% (S9) and highest boil-off loss value of

Table 1. Mean values of performance in parental breeds

Sl.No.	Breeds	Shell ratio(%)	Raw silk (%)	Boil-off loss (%)
Ovals				
1	CSR17	23.61	18.37	22.66
2	CSR27	25.01	18.40	22.33
3	JPN8	23.81	18.26	21.50
4	JPN7	23.65	18.15	21.90
5	S5	23.50	18.30	23.22
6	BBE226	23.33	17.09	25.04
	CD at 5%			**0.603**
Dumb-bells				
1	CSR16	22.18	18.12	23.09
2	CSR26	21.95	18.32	22.66
3	S9	21.72	19.22	22.01
4	D13	21.35	17.80	22.87
5	BBE247	22.04	16.35	25.05
6	BBE267	18.15	16.30	25.06
	CD at 5%			**0.923**

25.06% was recorded by BBE267 with values ranging from 22.01 to 25.26%. The least boil-off loss was registered by S9 (22.01%) followed by CSR26 (22.66%) and the maximum of 25.06% was recorded by BBE267 against the average of 23.61%.

Comparison of the mean values of shell ratio, raw silk percentage and boil-off loss exhibiting variations among the bivoltine single cross hybrids with a maximum shell ratio of 24.30% in CSR27xCSR26 and a minimum of 22.52% (JPN8xS9) and 21.29% in the control. The raw silk percentage ranged from 17.16% (CSR17xD13) to 19.08% in CSR27xCSR26. In boil-off loss trait, maximum of 24.89% in CSR17xBBE247 and a minimum of 23.25% in CSR27xCSR26 as against the 23.89% in the control hybrid, CSR2xCSR4, was recorded (Table 2).

Variation in shell ratio, raw silk percentage and boil-off loss among the bivoltine three-way cross (oval x oval) x dumb-bell hybrids (Table 3) (CSR27xCSR17)xBBE267 recorded lowest cocoon shell ratio of 19.87% and highest value of 22.31% by (JPN8xCSR17)xCSR26 was recorded. Raw silk percentage ranged from 16.54% (CSR27xCSR17)xBBE267 to 19.58% in

Table 2. Mean values of performance and heterosis in single cross hybrids

Sl .No	Hybrid	Performance			Heterosis (%)		
		Shell Ratio (%)	Raw Silk (%)	Boil-off loss (%)	Shell Ratio	Raw silk	Boil-off loss
1	CSR17xCSR16	23.14	17.38	24.31	0.72	2.26	6.82
2	CSR17xCSR26	23.22	19.01	24.00	-10.87	2.04	4.55
3	CSR17xS9	23.18	17.66	24.25	4.24	3.01	6.82
4	CSR17xD13	22.68	17.16	24.87	7.89	7.03	4.35
5	CSR17xBBE247	22.82	17.18	24.89	2.93	-0.08	4.73
6	CSR17xBBE267	22.77	18.75	24.03	-1.02	5.73	5.45
7	CSR27xCSR26	24.30	19.08	23.25	2.11	11.55	-1.43
8	CSR27xS9	23.56	18.75	24.03	2.09	2.18	0.01
9	CSR27xD13	23.51	18.39	23.83	1.41	4.13	-1.02
10	CSR27xBBE247	23.37	18.59	24.65	6.13	2.25	4.34
11	CSR27xBBE267	23.73	18.84	24.58	2.81	7.18	4.54
12	JPN8xS9	22.52	18.20	23.83	3.55	0.81	6.98
13	JPN8xD13	23.02	18.79	24.13	2.80	9.86	6.97
14	JPN8xBBE247	23.12	18.19	24.65	9.02	4.74	2.22
15	JPN8xBBE267	23.02	17.39	24.50	1.85	0.35	6.51
16	JPN7xD13	22.74	17.71	24.53	-3.41	10.57	1.15
17	JPN7xBBE247	23.05	17.33	24.50	6.86	-0.137	1.09
18	JPN7xBBE267	23.59	17.47	24.83	6.93	4.77	-0.36
19	S5xBBE247	23.35	17.63	24.27	4.63	3.94	4.26
20	S5xBBE267	22.88	17.34	24.07	2.20	0.46	2.17
21	BBE226xBBE267	22.59	17.47	24.68	10.83	7.91	-5.48
22	CSR2xCSR4 **(C)**	21.29	18.23	23.89	-2.04	3.34	-2.13

Table 3. Mean values of performance and heterosis in three-way cross hybrids

Sl. No	Hybrid	Performance			Heterosis (%)		
		Shell Ratio(%)	Raw silk (%)	Boil-off loss (%)	Shell Ratio	Raw silk	Boil-off loss
1	(CSR27xCSR17)xBBE267	19.87	16.54	24.51	-2.88	1.59	2.84
2	(CSR27xCSR17)xD13	20.23	16.87	24.17	-6.76	-1.91	4.73
3	(CSR27xCSR17)xS9	21.36	18.36	23.38	-8.43	-2.71	5.50
4	(CSR27xCSR17)xBBE247	21.50	17.22	24.31	-10.89	1.91	4.95
5	(CSR27xCSR17)xCSR16	21.17	18.89	23.72	-9.02	-0.75	3.75
6	(CSR27xCSR17)xCSR26	21.24	18.54	23.89	-2.92	1.90	4.10
7	(JPN8xCSR27)xD13	21.52	19.88	24.52	-4.93	-1.15	7.54
8	(JPN8xCSR27)xS9	21.87	18.59	24.08	0.47	1.06	6.64
9	(JPN8xCSR27)xBBE247	20.43	17.46	24.51	-6.20	-1.53	5.63
10	(JPN8xCSR27)xCSR16	20.78	17.52	24.25	-8.15	-4.97	6.50
11	(JPN8xCSR27)xCSR26	20.57	17.10	24.26	-7.95	-2.56	10.36
12	(JPN8xCSR17)xD13	21.58	18.24	23.83	-3.03	0.33	3.73
13	(JPN8xCSR17)xBBE247	20.33	16.62	24.54	-7.52	-4.62	7.27
14	(JPN8xCSR17)xCSR16	20.72	16.94	24.52	-4.52	-2.42	5.84
15	(JPN8xCSR17)xCSR26	22.31	19.58	22.51	0.28	0.49	1.09
16	(JPN8xJPN7)xBBE247	20.84	18.44	23.86	-6.01	-2.92	2.97
17	(JPN8xJPN7)xCSR16	21.32	19.23	23.81	-5.11	-2.58	2.95
18	(JPN8xJPN7)xCSR26	21.52	17.57	24.17	-9.07	-4.51	8.87
19	(S5xCSR27)xCSR16	20.60	19.30	23.97	-11.14	-1.70	6.63
20	(S5xCSR27)xCSR26	20.48	17.23	24.55	-7.06	-2.12	7.44
21	(S5xJPN8)xCSR26	21.41	18.53	24.19	0.28	1.68	7.47
22	CSR2xCSR4 **(C)**	20.91	17.47	23.22	0.88	1.07	2.04

(JPN8xCSR17)xCSR26. The least boil-off loss of 22.51% in (JPN8xCSR17)xCSR26 and highest of 24.55% in (S5xCSR27)xCSR26 was recorded. Some other hybrids have also shown lower deviation (<24.00%) against the highest of 24.55% recorded by (S5xCSR27)xCSR26 and control hybrid, CSR2 x CSR4 with 23.22%.

Mean values of performance in respect of shell ratio, raw silk percentage and boil-off loss in respect of bivoltine four-way cross hybrids is presented in Table 4. [(CSR27xCSR17)x(CSR16xCSR26)] exhibited least shell ratio (19.97%), raw silk percentage was less (16.49%) in [(JPN8xCSR17)x(D13xCSR16)] and minimum boil-off loss of 22.33% was recorded by [(JPN8xCSR17)x(D13xCSR26)]. The shell ratio of four-way cross hybrids ranged from 19.97% [(CSR27xCSR17)x(CSR16xCSR26)] to 28.89% [(JPN8xCSR17)x(D13xCSR26)]. Similarly, raw silk percentage ranged from 16.49% [(JPN8xCSR17)x(D13xCSR16)] to 19.52% [(JPN8xCSR17)x(D13xCSR26)]. Most of the four-way cross hybrids have shown less variation

in boil-off loss with the average ratio of 23.36% and ranged from 22.33 to 24.75%. The hybrid [(JPN8xCSR17)x(D13xCSR26)] has recorded lowest boil-off loss (22.33%) as compared to [(CSR27xCSR17)x(S9xCSR16)] with 23.75% and the control hybrid [(CSR2xCSR27)x(CSR6xCSR26)] with the ratio of 24.04%.

Table 4. Mean values of performance and heterosis in four-way cross hybrids

Sl. No	Hybrid	Performance			Heterosis (%)		
		Shell Ratio(%)	Raw silk (%)	Boil-off loss (%)	Shell Ratio	Raw silk	Boil-off loss
1	(CSR27xCSR17)x (CSR16xCSR26)	19.97	18.62	24.43	-2.88	1.59	6.22
2	(CSR27xCSR17)x (S9xCSR16)	20.14	18.87	23.75	-6.76	-1.91	4.40
3	(CSR27xCSR17)x (S9xCSR26)	21.60	18.10	23.69	-2.43	-2.71	4.13
4	(CSR27xCSR17)x (D13xCSR26)	21.51	19.34	23.51	4.22	4.48	2.17
5	(CSR27xCSR17)x(D13xS9)	19.40	18.89	23.47	-3.14	-0.75	3.16
6	(CSR27xCSR17)x(D13x CSR16)	19.93	17.12	23.52	-2.92	1.89	2.17
7	(JPN8xCSR27)x(S9xCSR16)	20.77	17.06	23.55	-5.46	-0.24	5.01
8	(JPN8xCSR27)x(S9xCSR26)	21.46	19.45	23.52	-0.96	2.69	5.36
9	(JPN8xCSR27)x(D13x CSR26)	21.56	19.18	23.50	-0.92	3.62	3.84
10	(JPN8xCSR27)x(D13xS9)	21.43	19.40	23.57	1.05	-4.97	5.00
11	(JPN8xCSR27)x(D13x CSR16)	20.55	19.12	23.65	-3.60	-2.56	3.84
12	(JPN8xCSR17)x(S9xCSR26)	22.01	19.05	22.45	-3.03	0.33	5.02
13	(JPN8xCSR17)x(D13x CSR26)	22.89	19.52	22.33	2.27	3.99	3.84
14	(JPN8xCSR17)x(D13xS9)	21.88	16.94	22.35	-4.52	-2.42	5.05
15	(JPN8xCSR17)x(D13x CSR16)	20.03	16.49	23.15	0.28	0.49	3.84
16	(JPN8xJPN7)x(D13xCSR26)	20.15	19.43	23.30	-6.10	-2.93	5.68
17	(JPN8xJPN7)x(D13xS9)	20.23	19.23	23.51	-5.11	-2.58	6.82
18	(JPN8xJPN7)x(D13xCSR16)	19.80	18.57	23.56	0.67	-4.49	5.62
19	(S5xCSR27)x(D13xS9)	21.73	19.18	23.49	-0.87	-1.70	3.30
20	(S5xCSR27)x(D13xCSR16)	21.16	17.85	23.52	-7.06	-2.12	2.21
21	(S5xJPN8)x(D13xCSR16)	19.87	18.87	23.50	0.40	2.17	3.84
22	(CSR2xCSR27)x (CSR6xCSR26) (C)	20.17	18.91	24.04	-8.50	2.09	4.75

Table 5. Rearing and reeling performance of bivoltine single cross hybrids
(Mean values of 3 seasons)

Sl. No.	Hybrid	Yield / 10000 larvae (No.)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Raw silk (%)	Filament length (m)	Reelability (%)	Neatness (p)	Boil-off loss ratio (%)	E. I. (%)
1	CSR17xCSR16	9555	1.984	0.459	23.14	17.38	978	84.14	92.50	24.31	53.81
2	CSR17xCSR26	9029	1.929	0.448	23.22	19.01	972	79.96	92.17	24.00	50.83
3	CSR17xS9	9183	1.989	0.461	23.18	17.66	1007	83.55	91.80	24.25	53.69
4	CSR17xD13	9458	1.904	0.432	22.68	17.16	936	79.85	91.98	24.87	49.43
5	CSR17xBBE247	9048	1.916	0.437	22.82	17.18	996	80.15	90.90	24.89	51.86
6	CSR17xBBE267	9612	1.915	0.436	22.77	18.75	977	81.75	91.72	24.03	49.21
7	CSR27xCSR26 *	9608	2.074	0.504	24.30	19.08	1098	88.05	93.75	23.25	59.16
8	CSR27xS9	9511	1.982	0.466	23.56	18.75	949	85.10	92.81	24.03	55.15
9	CSR27xD13	9661	1.948	0.458	23.51	18.39	1076	86.12	93.10	23.83	55.10
10	CSR27xBBE247	9141	1.823	0.426	23.37	18.59	882	80.10	91.33	24.65	48.71
11	CSR27xBBE267	9240	1.850	0.439	23.73	18.84	970	80.50	91.86	24.58	45.93
12	JPN8xS9	9204	1.922	0.433	22.52	18.20	1046	82.60	92.33	23.83	52.52
13	JPN8xD13	9286	1.876	0.432	23.02	18.79	1040	82.50	92.50	24.13	52.15
14	JPN8xBBE247	9498	1.881	0.435	23.12	18.19	882	80.50	90.96	24.65	45.72
15	JPN8xBBE267	9517	1.877	0.432	23.02	17.39	1062	81.80	92.10	24.50	50.26
16	JPN7xD13	9505	1.957	0.445	22.74	17.71	965	80.50	92.03	24.53	51.59
17	JPN7xBBE247	9378	1.879	0.433	23.05	17.33	900	80.10	91.80	24.50	49.22
18	JPN7xBBE267	9538	1.823	0.430	23.59	17.47	897	80.55	91.05	24.83	45.77
19	S5xBBE247	9256	1.812	0.423	23.35	17.63	924	80.50	92.10	24.27	48.01
20	S5xBBE267	9356	1.876	0.429	22.88	17.34	1025	82.00	92.06	24.07	52.27
21	BBE226xBBE267	9170	1.806	0.408	22.59	17.47	919	80.50	90.10	24.68	45.74
22	CSR2xCSR4 **(C)**	9065	1.855	0.395	21.29	18.23	943	80.00	92.00	23.89	45.97

* Selected hybrid.

Table 6. Rearing and reeling performance of bivoltine three-way cross hybrids

(Mean values of 3 seasons)

Sl. No.	Hybrid	Yield / 10000 larvae (No.)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Raw silk (%)	Filament length (m)	Reelability (%)	Neatness (p)	Boil-off loss ratio (%)	E. I. (%)
1	(CSR27xCSR17)xBBE267	8955	1.988	0.395	19.87	16.54	906	81.10	91.40	24.51	43.09
2	(CSR27xCSR17)xD13	8930	1.928	0.390	20.23	16.87	910	80.70	92.20	24.17	44.05
3	(CSR27xCSR17)xS9	9445	1.994	0.426	21.36	18.36	1001	86.05	93.00	23.38	50.08
4	(CSR27xCSR17)xBBE247	8893	1.786	0.384	21.50	17.22	899	79.55	91.20	24.31	44.38
5	(CSR27xCSR17)xCSR16	8944	1.866	0.395	21.17	18.89	952	83.20	91.50	23.72	43.95
6	(CSR27xCSR17)xCSR26	9445	1.996	0.424	21.24	18.54	1008	86.20	92.00	23.89	49.80
7	(JPN8xCSR27)xD13	8877	1.863	0.401	21.52	19.88	1021	86.12	92.10	24.52	50.10
8	(JPN8xCSR27)xS9	9423	1.948	0.426	21.87	18.59	1090	87.10	93.35	24.08	51.99
9	(JPN8xCSR27)xBBE247	8801	1.943	0.397	20.43	17.46	927	84.10	91.50	24.51	46.88
10	(JPN8xCSR27)xCSR16	8859	1.934	0.402	20.78	17.52	991	80.40	91.20	24.25	44.58
11	(JPN8xCSR27)xCSR26	8795	1.974	0.406	20.57	17.10	1005	84.80	91.50	24.26	48.27
12	(JPN8xCSR17)xD13	9448	1.867	0.403	21.58	18.24	1007	83.10	92.00	23.83	48.95
13	(JPN8xCSR17)xBBE247	8916	1.860	0.378	20.33	16.62	903	82.10	91.50	24.54	46.94
14	(JPN8xCSR17)xCSR16	8782	1.897	0.393	20.72	16.94	947	79.95	91.60	24.52	42.87
15	(JPN8xCSR17)xCSR26*	9466	1.901	0.424	22.31	19.58	1098	87.50	93.50	22.51	52.96
16	(JPN8xJPN7)xBBE247	8952	1.867	0.389	20.84	18.44	922	80.50	91.50	23.86	43.17
17	(JPN8xJPN7)xCSR16	8898	1.857	0.396	21.32	19.23	953	80.90	91.60	23.81	43.79
18	(JPN8xJPN7)xCSR26	8834	1.873	0.403	21.52	17.57	947	80.50	91.50	24.17	44.14
19	(S5xCSR27)xCSR16	8892	1.927	0.397	20.60	19.30	927	80.40	91.00	23.97	43.69
20	(S5xCSR27)xCSR26	8807	1.943	0.398	20.48	17.23	936	80.10	92.10	24.55	43.84
21	(S5xJPN8)xCSR26	9032	1.948	0.417	21.41	18.53	990	85.50	92.20	24.19	49.53
22	CSR2xCSR4 (C)	9065	1.889	0.395	20.91	17.47	943	84.50	93.80	23.22	49.81

* Selected hybrid.

Table 7. Rearing and reeling performance of bivoltine four-way cross hybrids
(Mean values of 3 seasons)

Sl. No.	Hybrid	Yield / 10000 larvae (No.)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Raw silk (%)	Filament length (m)	Reelability (%)	Neatness (p)	Boil-off loss ratio (%)	E. I. (%)
1	(CSR27xCSR17)x (CSR16xCSR26)	9178	2.093	0.418	19.97	18.62	993	80.10	91.89	23.43	43.83
2	(CSR27xCSR17)x (S9xCSR16)	9274	2.120	0.427	20.14	18.87	1073	83.30	91.67	23.75	45.76
3	(CSR27xCSR17)x (S9xCSR26)	9341	2.162	0.467	21.60	18.10	1108	85.20	92.33	23.69	48.54
4	(CSR27xCSR17)x (D13xCSR26)	9324	2.143	0.461	21.51	19.34	1112	86.00	92.44	23.51	49.22
5	(CSR27xCSR17)x (D13xS9)	9356	2.170	0.421	19.40	18.89	1120	86.20	92.40	23.47	49.09
6	(CSR27xCSR17)x (D13xCSR16)	9460	2.072	0.413	19.93	17.12	1102	84.00	91.67	23.52	47.14
7	(JPN8xCSR27)x (S9xCSR16)	9230	2.176	0.452	20.77	17.06	1049	85.50	92.22	23.55	49.69
8	(JPN8xCSR27)x (S9xCSR26)	9381	2.143	0.460	21.46	19.45	1092	86.00	92.30	23.52	50.60
9	(JPN8xCSR27)x (D13xCSR26)	9330	2.180	0.470	21.56	19.18	1118	86.40	92.70	23.50	49.88
10	(JPN8xCSR27)x(D13xS9)	9536	2.156	0.462	21.43	19.40	1066	79.55	92.34	23.57	47.73
11	(JPN8xCSR27)x (D13xCSR16)	9157	2.034	0.418	20.55	19.12	1039	79.80	91.67	23.65	43.13
12	(JPN8xCSR17)x (S9xCSR26)	9381	2.144	0.472	22.01	19.05	1100	84.50	92.67	22.45	50.67
13	(JPN8xCSR17)x (D13xCSR26) *	9447	2.153	0.494	22.89	19.52	1168	88.55	93.40	22.33	56.47

Sl. No.	Hybrid	Yield / 10000 larvae (No.)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Raw silk (%)	Filament length (m)	Reelability (%)	Neatness (p)	Boil-off loss ratio (%)	E. I. (%)
14	(JPN8xCSR17)x(D13xS9)	9365	2.148	0.470	21.88	16.94	1105	86.00	92.33	22.35	50.58
15	(JPN8xCSR17)x (D13xCSR16)	9195	2.077	0.415	20.03	16.49	1021	80.40	91.67	23.15	45.08
16	(JPN8xJPN7)x (D13xCSR26)	9158	2.144	0.432	20.15	19.43	1026	80.00	92.44	23.30	44.88
17	(JPN8xJPN7)x(D13xS9)	9136	2.086	0.422	20.23	19.23	1033	80.10	91.87	23.51	44.30
18	(JPN8xJPN7)x (D13xCSR16)	9204	2.121	0.420	19.80	18.57	1012	81.20	91.60	23.56	47.88
19	(S5xCSR27)x(D13xS9)	9326	2.172	0.472	21.73	19.18	1099	83.50	92.20	23.49	48.76
20	(S5xCSR27)x(D13xCSR16)	9191	2.123	0.428	20.16	17.85	1040	80.50	91.80	23.52	45.92
21	(S5xJPN8)x(D13xCSR16)	9256	2.179	0.433	19.87	18.87	1020	84.00	91.40	23.50	49.32
22	(CSR2xCSR27)x (CSR6xCSR26) (C)	9155	2.043	0.412	20.17	18.91	996	86.20	92.10	24.04	49.15

* Selected hybrid.

The heterosis values of shell ratio, raw silk percentage and boil-off loss in single, three-way and four-way cross hybrids are presented in Table 2, 3 and 4. In respect of shell ratio and raw silk percentage, among single cross hybrids (Table 2) highest shell ratio heterosis value of 10.83% was registered by BBE226xBBE267, in raw silk percentage CSR27xCSR26 scored top heterosis of 11.55%. Among three-way cross hybrids, maximum shell ratio heterosis of 0.476% was recorded by (JPN8xCSR27)xS9, raw silk percentage 1.91% by (CSR27xCSR17)xBBE247. Four-way cross hybrid (CSR27xCSR17)x(D13xCSR26) recorded highest shell ratio (4.22%) and raw silk percentage (4.48%) heterosis values.

In respect of the boil-off loss ratio heterosis, it is observed that, four single cross hybrids and control hybrid have recorded negative heterosis, which is desirable for this trait (Table 2). Among the single cross hybrids, negative heterosis values ranged from -0.36% in JPN7xBBE267 followed by CSR27xD13 (-1.02%), CSR27xCSR26 (-1.43%) and BBE226xBBE267 (-5.48%).

Heterosis in respect of boil-off loss recorded in three-way and four-way cross hybrid combinations is as in Table 3 and 4. The three-way cross hybrids have recorded positive heterosis ranging from 1.09% (JPN8xCSR17)xCSR26 to 10.36% (JPN8xCSR27)xCSR26. Among the four-way cross hybrids, expressing positive boil-off loss heterosis, the resultant values ranged from 2.17% [(CSR27xCSR17)x(D13xCSR26)] to 6.82% (JPN8xJPN7)x(D13xS9).

The rearing and reeling performance of the single, three-way and four-way cross hybrids presented in Table 5, 6 and 7 indicate significant difference for all the economically important quantitative traits such as yield/10000 larvae, cocoon weight., shell weight., shell ratio, raw silk percentage, filament length, reelability, neatness and boil-off loss ratio. Multiple trait evaluation index (E.I.) estimated including all the said quantitative traits to select the promising hybrids specify that, from the commercial point of view, one hybrid combination of each with highest E.I. value are the best compared to the other hybrids in different cross hybrids.

DISCUSSION

The outcome of silkworm breeding is judged by the best desirable traits of the parental characters that appear in F1 hybrids. Similarly, from the reeling point of view, cocoon shell ratio, raw silk percentage, boil-off loss ratio and other quantitative traits that are directly linked to raw silk production are considered as the most important ones. In sericulturally advanced countries, the silkworm breeders have developed productive parental breeds and hybrids with low boil-off loss ratio and quality silk (Kurasawa, 1968, Gamo and Hirabayashi, 1983). During the course of breeding process, the boil-off loss with reference to cocoon shell has been given utmost importance along with other quantitative and qualitative traits (Harada et al., 1961, Gamo and Ichiba, 1971, Yokoyama, 1959, Mano et al., 1988). Analyzed data on the performance of parents and hybrids in expression of boil-off loss corroborate with the earlier findings of Sonwalker (1969), Sidhu and Sonwalker (1969), Sinha et al., (1992) and Raghavendra Rao et al., (2004).

In the present study, the oval breeds registered higher average of boil-off loss ratio (22.78%) as compared to dumb-bell breeds (23.61%). However, the hybrids have registered intermediate values (24.46%) in single cross, (24.01%) three-way cross and (23.36%) four-way cross hybrids as compared to their parents. Among the oval breeds, JPN8 (21.50%) and JPN7 (21.90%) recorded the lower values, single cross hybrid CSR27xCSR26 (23.25%), three-way cross hybrid (JPN8xCSR17)xCSR26 (22.51%) and four-way cross hybrid (JPN8xCSR17)x(D13xCSR26) (22.33%) have recorded boil-off loss less than 24%. These results are in conformity with findings of Gamo and Hirabayashi (1983), Sinha et al. (1992), Basavaraja et al., (2000) and Seetharamulu et al., (2013).

The phenomenon of heterosis in conjunction with the expression of boil-off loss ratio analyzed in the hybrids under present study facilitated procedures to identify the promising hybrids. Further, the more uniformity in the expression of this trait in hybrids than the parents is one of the desirable features to understand the genetic constitution of the hybrids for their commercial exploitation as evidenced by the mean values computed for this trait. For boil-off loss ratio trait, a negative heterosis value is desirable corroborate with the earlier findings of Basavaraja et al., (2000), Raghavendra Rao et al., (2004) and Seetharamulu et al., (2013). For instance, high magnitude of negative heterosis was recorded in the combinations of BBE226xBBE267 (-5.48%) and CSR27xCSR26 (-1.43%) which could be attributed to the higher mid parental value. The heterosis expressed is variable in different combinations / crosses of hybrids and the results are in accordance with the findings of Gamo and Hirabayashi (1983). In certain hybrids of different crosses, remarkably less heterosis was noticed and very often the hybrids were intermediate between parents for this trait. The manifestation of heterosis phenomenon was explained by over-dominance hypothesis by Jones (1917), Mather (1955) and Fisher (1965). The result of the present study indicated the manifestation of heterosis in different hybrid combinations for this trait. It is possible that, the theories put forward are not mutually extensive as it could be done to several factors like dominance, incomplete dominance, epistasis and maternal effect responsible for the expression of heterosis in varying degrees and support the views of Bowman (1959).

Positive correlation that existed between boil-off loss ratio and cocoon shell weight of parental breeds clearly confirms the findings of Gamo and Hirabayashi (1983). Improvement of boil-off loss ratio towards low value can be achieved through selection by choosing the crossing types exhibiting higher negative heterosis results for this trait which is in accord with the findings of Basavaraja et al., (2000) and Seetharamulu et al., (2013).

Improvement of boil-off loss ratio towards low value can be achieved through selection by choosing different crossing pattern showing higher negative heterosis for boil-off loss trait. On the basis of hybrids rearing and reeling performance with multiple trait evaluation index values and heterosis with particular reference to different quantitative traits including boil-off loss ratio, the hybrids viz., CSR27xCSR26 (single-cross), (JPN8xCSR17)xCSR26 (three-way cross) and (JPN8xCSR17)x(D13xCSR26) four-way cross hybrids were identified as promising

ones deserving further commercial exploitation. Also, outcome results of the present study makes it clear that evaluation of boil-off loss ratio among the bivoltine breeds / hybrids identified will enhance qualitative merit of raw silk.

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Ovicidal and adulticidal effect of acaropathogenic fungi, neem oil and new acaricide molecules on *Tetranycus urticae* Koch

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ABSTRACT: The relative toxicity of two acaropathogenic fungi (*Hirsutella thompsonii* and *Beauveria bassiana*), neem oil and three new acaricide molecules *viz*., fenazaquin 10 EC, spiromesifen 240 SC and diafenthiuron 50 WP to two-spotted spider mites (egg and adults) were evaluated against a standard check and untreated control under laboratory conditions. 24 hours after treatment, fenazaquin 10 EC excelled in ovicidal activity with a mean egg mortality of 40.81 per cent. The next best treatment was spiromesifen 240 SC which recorded 15.17 per cent egg mortality. Both fenazaquin 10 EC and diafenthiuron 50WP exhibited 100 per cent adult mortality within 24 hours of treatment application. After 72 hours, all the treatments except *B. bassiana* caused significantly high egg mortality. Neem oil (41.00 per cent) and *H. thompsonii* (31.98 per cent) emerged as the next best candidates with respect to adult mortality, while spiromesifen 240 SC recorded the lowest adult mortality of 3.40 per cent.

KEYWORDS: Bioassay; *Tetranychus urticae*; fenazaquin 10 EC; spiromesifen 240 SC; diafenthiuron 50 WP; *Hirsutella thompsonii*; *Beauveria bassiana*; neem oil

Two spotted spider mite (TSSM), *Tetranychus urticae* Koch is a highly polyphagous pest of numerous vegetable crops. Okra is one of the major vegetables cultivated in India throughout the year. One of the limiting factors in the cultivation of okra is the incidence of mite pest, *T. urticae,* especially during summer season which causes severe damage and yield loss up to 17.5 per cent (Ghosh *et al.,* 1996). The intense use of synthetic chemicals against this pest has

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resulted in the development of resistance to a wide range of chemicals. This has necessitated the development of newer chemicals with novel modes of action. There is also an increasing interest for natural pesticides which are derived from plants and micro organisms, since they are perceived to be safer than the synthetic chemicals (Yaner *et al*., 2011). The objective of the study was to compare the ovicidal and adulticidal effects of two acaropathogenic fungi(*Hirsutella thompsonii* and *Beauveria bassiana)*, neem oil and new acaricide molecules along with a standard check (fenazaquin 10 EC, spiromesifen 240 SC, diafenthiuron 50 WP and dicofol 18.5 EC) and untreated control on *T. urticae*, which may be considered as a study for developing suitable IPM for *T. urticae*.

Bioassay studies on *T. urticae* were conducted in the Acarology laboratory, Department of Agricultural Entomology, College of Horticulture, Vellanikkara at a temperature of 30 ± 3^0C and 58 ± 1.4 % relative humidity.

The stock culture of *T. urticae* was established in polyhouse on potted plants of okra. To obtain fixed age eggs/females of *T. urticae,* mites from the stock culture were also reared on okra leaves with their upper surface down on wet cotton bed in *Petri* plates in the laboratory at 30 ± 2^0C and 58 ± 1.4 % relative humidity. The culture was observed daily and the leaves were changed periodically.

Topical application method was employed to study the ovicidal effect of two acaropathogenic fungi, neem oil and three new acaricide molecules along with a standard check and untreated control on *T. urticae* (Table 1).

To obtain *T. urticae* eggs of uniform age, gravid females were taken from the mite culture with the help of a moistened zero size camel hair brush and kept individually on okra leaf discs (2 cm^2) placed underside up in p*etri* plates with wet cotton pad. The females, 24 hours after oviposition were subsequently removed for performing the bioassay on eggs. Leaf discs containing *T. urticae* eggs of uniform age were sprayed with the treatments to be tested using a hand atomizer (2 ml/disc) untreated control, sprayed with water. Dicofol 18.5 EC was used as the standard check. The treated leaf discs with eggs were air dried at room temperature and placed in Petri plates. All treatments were replicated three times. Observations on mortality of eggs were recorded at 24, 48 and 72 hours intervals with a stereo binocular microscope. The per cent mortality values were then corrected for control mortality using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality (\%)} = \frac{\{\text{Test mortality (\%)} - \text{Control mortality (\%)}\}}{100 - \text{Control mortality (\%)}} \times 100$$

In adulticidal bioassay leaf dip bioassay method was employed to study the effect of different treatments listed in Table 1. Leaf discs of 2 cm^2 diameter, were dipped in aqueous solution of prepared concentration of the acaricides for ten seconds and then air dried for completely evaporating the water droplets. Ten gravid females of uniform age taken from the stock

Table 1. Ovicidal and Adulticidal effects of different treatments on *T. urticae* in okra under laboratory conditions

Sl. No	Treatments& dosage used	Corrected Mortality (%) over untreated control water spray					
		Ovicidal effect			Adulticidal effect		
		24H	48H	72H	24H	48H	72H
1	Fenazaquin 10 EC – dosage 25 ì L /10 ml	40.81 [a]	51.79 [a]	94.21 [a]	100 [a]	100 [a]	100 [a]
2	Spiromesifen 240 SC – dosage 8 ì L/ 10 ml	15.17 [b]	34.49 [bc]	90.21[a]	0	0	3.40 [b]
3	Diafenthiuron 50 WP @ 400g ai/ha – dosage 16mg/10 ml	4.18 [d]	39.79 [b]	94.88[a]	100 [a]	100 [a]	100 [a]
4	Standard Check (Dicofol 18.5 EC @250g ai/ha) –dosage 25 ì L/10 ml	11.16 [bc]	32.12[bc]	70.54[a]	100 [a]	100 [a]	100 [a]
5	Neem oil 2% - dosage 200 ì L /10 ml	1.51 [d]	21.66 [c]	67.55[a]	37.67 [b]	37.67 [b]	41.00 [a]
6	*Beauveria bassiana* @ 10^7 spores/ml - dosage 100 mg/10 ml	0.04 [d]	3.59 [d]	7.29 [b]	0	0	6.73 [b]
7	*Hirsutella thompsonii* (@ 10^7 spores/ml) –dosage 100 mg/10 ml	2.11 [d]	37.45 [bc]	86.88 [a]	0	0	31.98 [a]

Means followed by same letters do not differ significantly by DMRT (p = 0.05)

culture, were released on to the treated leaf disc kept on wet cotton pad in Petri plate. Such three plates were maintained for each treatment. Leaf discs dipped in only water served as control, while those dipped in Dicofol 18.5 EC served as standard check. Observations on mortality of adult mites were recorded at 24, 48 and 72 hours interval using a stereo binocular microscope and per cent mortality was calculated. The values were then corrected for control mortality using Abbott's formula (Abbott, 1925).

Data corrected for mortality in the control using Abbott's formula were after square root transformation subjected to one- way analysis of variance ($P < 0.05$). Means were compared by Duncan's Multiple Range Test (DMRT) to determine significant differences at $P < 0.05$.

Ovicidal bioassay: It is evident from the table that the ovicidal activity of all the treatments increased from 24h to 72h. 24 hours after treatment, fenazaquin 10 EC excelled in ovicidal

activity with a mean egg mortality of 40.81 per cent. The next best treatment was spiromesifen 240 SC which showed 15.17 per cent egg mortality. It was statistically on par with dicofol (11.16 %). Per cent mortality in diafenthiuron 50 WP (4.18), neem oil 2% (1.51), *B. bassiana* (0.04) and *H. thompsonii* (2.11) indicated that they were on par with each other though inferior to dicofol. 48 hours after treatment, fenazaquin continued to be the better treatment causing 51.79 per cent egg mortality. It was followed by diafenthiuron, *H. thompsonii* and spiromesifen with a mean mortality of 39.79, 37.45 and 34.49 respectively. These treatments were also on par with the standard check. After 72 hours, all the treatments except *B. bassiana* caused significantly high egg mortality ranging from 70.54 to 94.88 per cent. *B. bassiana* proved ineffective on eggs of *T. urticae* .

Adulticidal bioassay

The new acaricide molecules, fenazaquin 10 EC and diafenthiuron 50WP caused complete adult mortality within 24 hours of treatment application. Both the chemicals were superior to other treatments and were on par with the standard check, dicifol 18.5 EC, which also caused cent per cent adult mortality within 24 hours time. The next best treatment was neem oil 2% with a mean mortality of 37.67 per cent. However no adult mortality was observed with adults exposed to spiromesifen 240 SC and acaropathogenic fungi *viz*., *B. bassiana* and *H. thompsonii* up to 48 hours of exposure. After 72 hours of application, neem oil (41 per cent) and *H. thompsonii* (31.98 per cent) emerged as the next leading treatments. *B. bassiana* and spiromesifen were found inferior with adult mortality of less than ten per cent (Table 1).

Fenazaquin is an acaricide which belongs to quinazoline class of chemicals which inhibits mitochondrial electron transport (MET) at complex I. It has high efficacy against eggs and motile stages of tetranychid mites (Marcic *et al*., 2011). The high level of egg and adult mortality exhibited by this chemical, in a very short period of exposure was also earlier reported by many workers. In a laboratory bioassay conducted to test the ovicidal activity against *T.urticae* on okra, Sangeetha and Ramaraju (2013) reported 81.25 per cent egg mortality for fenazaquin 10 EC at 125 g a.i. ha^{-1}. 90.52 per cent mortality of adults of *T. macfarlanei* was caused by Fenazaquin 10 EC (Patil, 2005).

The insecto-acaricide diafenthiuron, is a novel thiourea compound that disrupts oxidative phosphorylation by inhibition of the mitochondrial ATP synthase enzyme. It has been reported as effective against active stages of spider mites (Marcic *et al*., 2011). Similar results were also reported by Patil (2005) who found that use of diafenthiuron resulted in more than 96 per cent mortality of adult mites. The ovicidal activity of diafenthiuron was identified by Patil and Nandihalli (2007) who, based on their bioassay studies on *T. macfarlanei* infesting brinjal, reported that diafenthiuron caused more than 98 per cent egg mortality. The findings of the present study are in agreement with the above studies.

Spiromesifen, a tetronic acid derivative acts as inhibitor of acetyl-CoA-carboxylase, a key enzyme in fatty acid biosynthesis. It is highly toxic to eggs and immature stages of spider

mites, while it acts more slowly against adult females, causing reduction in fertility and fecundity (Marcic *et al*., 2011). In baseline susceptibility studies conducted by Nauen *et al*. (2005), spiromesifen did not have a marked effect against *T. urticae* adult females, but was highly toxic against eggs of the mite. Sato *et al*. (2011) observed that among the different developmental stages studied, the egg stage of *T. urticae* was found to be the most sensitive to spiromesifen. In the present study also spiromesifen recorded a higher reduction in egg count over untreated control compared to standard check, dicofol**.** Saryazdi *et al*. (2013) observed ovicidal activity as well as reduction in the survival rate, fecundity and egg hatching rate when spiromesifen was used. This peculiar growth regulatory effect of spiromesifen might be the reason for very low adult mortality as observed in the present study.

The acaricidal action of neem oil 2 per cent may be attributed to slow action of azadirachtin, which includes complete or partial antifeedant response, delayed and/or disrupted moulting and inhibited reproduction (Copping and Duke, 2007). The studies on spider mites indicate that azadirachtin, in addition to being toxic to various development stages, acts as antifeedant, reduces fecundity and fertility and shortens the life span of adult mite (Sundaram and Sloane, 1995). Umamahesheswari *et al*. (1999) noticed that among the different neem formulations and castor oil tested for their efficacy against red spider mite, following dip method in the laboratory, neem oil gave significantly higher mortality (79.60 per cent) compared to the other treatments tested. A moderate to high level of mortality was also observed in the present study.

Though *B. bassiana* has been reported as a promising fungal pathogen against spider mites by several workers, in the present study it was found inferior including *H. thompsonii*. This may be because this fungus require more time for its development to finally cause mycosis to the mites. The better performance of *H. thompsonii* may be related to the high relative humidity prevailed during the study which causes increased rate of infection (Gerson *et al.,*1979). The findings of Aghajanzadeh *et al*. (2006) also proves the high virulence of this fungus against *T. urticae*. In the present study *H. thompsonii* was also found to have high ovicidal than adulticidal activity.

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